Análisis espacio-temporal de los vasos primaverales de *Quercus robur* L. y *Quercus pyrenaica* Willd.: Respuesta al estrés climático en la transición atlántico-mediterránea

Por

Manuel Souto Herrero

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A mis niños, Manu y Adriana
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CONTENIDOS

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ABREVIATURAS

Siglas	Definición
r1	Vasos de la primera hilera
nr1	Vasos no pertenecientes a la primera hilera
RW	Anchura total del anillo
EW	Anchura de la madera temprana
LW	Anchura de la madera tardía
NV	Número total de vasos o poros
KS	Conductividad específica
TVA	Área total del lumen de los vasos
MVA	Área media del lumen de los vasos
M90	Área media del lumen de los vasos mayores del 90% del anillo
HYD	Diámetro ponderado hidráulicamente
SPEI	Índice Estandarizado de Precipitación-Evapotranspiración
NAO	Oscilación del Atlántico Norte
SIFAU	Sistema de Fotografía Automático
CRU	Climate Research Unit

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Introducción

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1 INTRODUCCIÓN GENERAL

La dendrocronología es una disciplina basada en el estudio de los anillos de crecimiento de los árboles, considerados como registros permanentes de las condiciones ambientales (Fritts & Swetnam 1989). La posibilidad de datar la edad de los anillos con precisión anual (Speer 2010) hace de ella una herramienta muy útil para llevar a cabo reconstrucciones del pasado reciente a través del análisis de los patrones espaciales y temporales de procesos biológicos, físicos o culturales.

La mayoría de los estudios dendrocronológicos estudian habitualmente la anchura del anillo para establecer las relaciones entre crecimiento y clima (Matisons & Brumelis 2012; Rozas et al. 2015) y obtener así información ecológica relevante, o utilizan los anillos como *proxies* para reconstrucción climática (Briffa et al. 2002a; Briffa et al. 2002b). Pero la anchura del anillo es una variable que a menudo aporta información muy limitada ya que integra los sucesos acaecidos a lo largo de toda la estación de crecimiento en un único *proxy* y no suele mostrar buena respuesta al clima en áreas donde éste no es limitante. La medición por separado de madera temprana y tardía en especies de anillo poroso supone una ligera mejora en este aspecto. Aunque presenta pocos problemas metodológicos (Blank 1997), su uso no es una práctica habitual y en muchos casos ofrece poca información adicional ya que normalmente la madera tardía y la anchura total presentan prácticamente la misma variación interanual (Nola 1996; García González 2000). Por ello, uno de los principales campos de investigación en la dendrocronología moderna es la búsqueda de nuevas variables que sirvan como descriptores alternativos de las relaciones clima-crecimiento.

El estudio de las características anatómicas de la madera se ha convertido en los últimos años en una nueva herramienta con gran potencial que permite obtener información ecológica en áreas donde la dendrocronología clásica está generalmente limitada (Fonti & García-González 2008; Fonti et al. 2010). Además de su bajo coste económico en comparación con otras técnicas como los análisis de isótopos estables (Robertson et al. 2008), ofrecen una mayor resolución temporal a nivel intraanual (Corcuera et al. 2004; García-González & Fonti 2006) y una relación mucho más directa con los procesos fisiológicos del árbol (Fonti et al. 2010). Varios autores han obtenido buenas relaciones entre el clima y el tamaño de los vasos conductores, especialmente para los grandes vasos primaverales de las especies de anillo poroso como las del género *Quercus*. Se observó una estrecha relación con las condiciones ambientales durante el momento de su formación (García-González & Eckstein 2003; Fonti & García-González 2008), pero también con la estación de crecimiento previa (Matisons & Brumelis 2012; González-González et al. 2014) o con la dormición (González González et al. 2015), probando que el tamaño de los vasos primaverales de las especies de anillo poroso puede ser empleado como un valioso *proxy* en reconstrucciones climáticas (Eckstein 2004; Fonti et al. 2010), especialmente en regiones templadas. Por otro lado, en las especies de anillo poroso los primeros elementos conductores del leño temprano son de gran tamaño y se mantienen funcionales durante una única estación de crecimiento (Cochard & Tyree 1990), siendo responsables de la conducción de la mayor parte del volumen de agua de la planta (Ellmore & Ewers 1985). El número y las dimensiones de los elementos conductores determinan la capacidad de conducción de agua y nutrientes, así como la vulnerabilidad frente al estrés hídrico (Tyree & Zimmermann 2002). Por lo tanto, las variables anatómicas permiten estudiar de una forma más rigurosa la adaptabilidad de las especies arbóreas a las condiciones hídricas (Carlquist 1975; Lachenbruch & McCulloh 2014).

Los ecosistemas forestales dominados por quercíneas de anillo poroso, extendidos por gran parte de Europa y abundantes en la Península Ibérica, incluyen especies de lento crecimiento que se instalan sobre suelos más o menos evolucionados, tendiendo a formar bosques densos y estables que muchas veces tienen carácter climácico (Bary-Lenger & Nebout 1993). En la Península Ibérica varios robles nemorales caducifolios europeos (*Quercus robur* L., *Q. petraea* (Matt.) Liebl.) presentan su límite suroccidental de distribución hacia la región Mediterránea. En estas áreas de transición a menudo

coexisten con otras especies de robles marcescentes (*Quercus pyrenaica* Willd., *Q. faginea* Lam., *Q. pubescens* Willd., *Q. canariensis* Willd. o *Q. lusitanica* Lam.), considerados como "submediterráneos" por su mayor tolerancia a condiciones de estrés hídrico. Este es el caso de Galicia, donde *Q. robur* constituye la especie dominante (Figura 1a), siendo progresivamente reemplazada por *Q. pyrenaica* (Figura 1b) bajo condiciones de mayor mediterraneidad y de aumento de la sequía estival (Sánchez de Dios et al. 2009). De hecho, *Q. pyrenaica* llega a formar masas prácticamente monoespecíficas en las zonas transicionales del interior de la región (Díaz-Maroto et al. 2007).

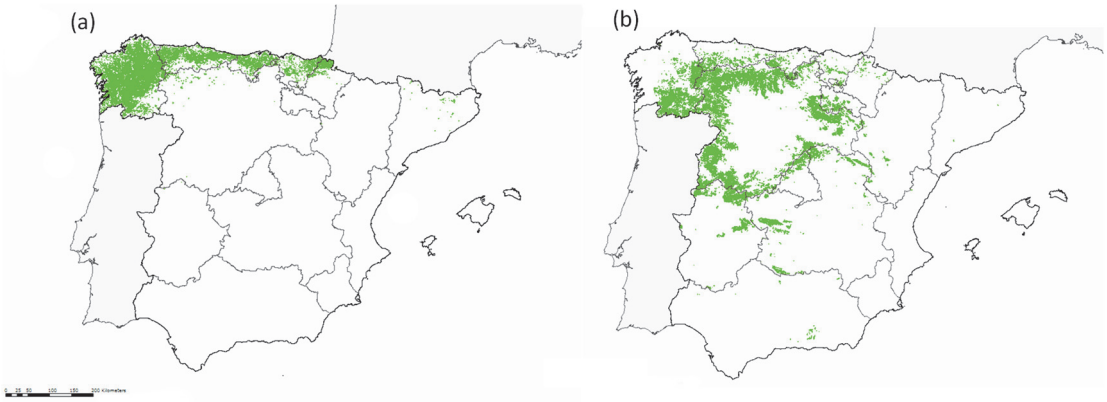

Figura 1. Mapas de distribución de *Quercus robur* (a) y *Quercus pyrenaica* (b) en España (Fuente: http://iniagis.inia.es/)

En la Península Ibérica no se comenzó a trabajar con los robles desde un punto de vista dendrocronológico hasta mediados de los años noventa, existiendo en la actualidad diversas cronologías que han sido desarrolladas en Galicia (Pérez Antelo & Fernández Cancio 1995; García González 2000; Rozas et al. 2009b; Rozas & García-González 2012a, b), Ancares leoneses (Domínguez-Delmás et al. 2003), Asturias y Cantabria (Rozas 2005; González-González et al. 2014) y el área mediterránea ibérica (Gea-Izquierdo & Cañellas 2014). Los primeros trabajos dendrocronológicos en Galicia estaban orientados a la obtención de cronologías largas con el fin de realizar reconstrucciones de variables climáticas a partir de la anchura de los anillos de crecimiento (Creus et al. 1995; Pérez Antelo & Fernández Cancio 1995). Posteriormente, García González (2000) estudió las principales variables climáticas que influyen sobre el crecimiento de *Q. robur* en el norte de Galicia, empleando cronologías de madera temprana y tardía, e identificó la disponibilidad de agua durante los meses de verano como principal factor limitante. No obstante, su papel sólo era claro en las zonas más bajas, próximas a la costa. Otras áreas de Galicia no mostraron una respuesta clara al clima, no siendo posible identificar las principales variables que controlaban el crecimiento radial. Para aquellas que mostraron una clara respuesta, ésta era en general inestable en el tiempo, y por tanto la reconstrucción de variables meteorológicas podría resultar inviable. Posteriormente, Rozas et al. (2009a) encontraron que existe una gran heterogeneidad geográfica en la variación interanual del crecimiento de *Q. robur* en Galicia, relacionada probablemente con la topografía y con el clima local.

En los últimos diez años, la aplicación de los sistemas automáticos de análisis de imagen y de determinadas herramientas informáticas al estudio de la anatomía cuantitativa de la madera ha favorecido el desarrollo de este campo de investigación, apareciendo numerosos trabajos basados en la medición de vasos de especies de poro difuso (Campelo et al. 2010; Arbellay et al. 2012; Abrantes et al. 2013; Oladi et al. 2014). Sin duda los mejores resultados han sido obtenidos estudiando los elementos conductores primaverales de *Quercus robur* (García-González & Eckstein 2003; Matisons & Brumelis 2012; Hroš & Vavrčík 2014; Kniesel et al. 2015), *Q. petraea* (González-González et al. 2014), *Q. pyrenaica* (Ballesteros et al. 2010; González-González et al. 2014; González González et al. 2015), *Q. faginea* (Corcuera et al. 2004) o *Q. macrocarpa* Michx. (St George et al. 2002), pero también en otras como *Castanea sativa* Miller (Fonti et al. 2007). Recientemente también se ha abordado el estudio de los vasos

de los robles desde el punto de vista de la xilogénesis, ayudando a comprender mejor los mecanismos implicados en su formación y las relaciones existentes con la fenología, tanto en *Q. robur* y *Q. pyrenaica* (Sass-Klaassen et al. 2011; González-González et al. 2013; Pérez-de-Lis et al. 2015), como en otras quercíneas de anillo poroso como *Q. mongolica* (Takahashi et al. 2013).

En la mayoría de los casos se ha puesto de manifiesto la existencia de una clara relación entre las condiciones ambientales y el tamaño de los vasos, bien mediante un mecanismo aparentemente directo (García-González & Eckstein 2003) o bien indirectamente a través de ciertos procesos fisiológicos (Fonti et al. 2007). En muchas ocasiones se aprecian respuestas a la precipitación, tanto durante la estación de crecimiento (Fonti & García-González 2008) como en la estación anterior (Matisons & Brumelis 2012; González-González et al. 2014), pero en otras también la temperatura parece desempeñar un papel importante en la formación de los vasos (Fonti et al. 2010). La respuesta climática de los vasos xilemáticos presenta mayor estabilidad a lo largo del tiempo que la de la anchura de los anillos (García-González & Eckstein 2003). Además se ha demostrado que es posible maximizar la respuesta a una determinada variable si se seleccionan los vasos de primavera de un modo adecuado (García-González & Fonti 2006; González-González et al. 2014). Por lo tanto, la dendrocronología aplicada a variables anatómicas cuantitativas de la madera puede aportar una valiosa información al conocimiento de las relaciones entre el crecimiento, la productividad y la salud de los árboles y el ambiente en que se desarrollan. En cualquier caso, son necesarios numerosos trabajos sobre distintas especies de árboles creciendo en diferentes ambientes para un conocimiento más detallado sobre las relaciones entre la anatomía de la madera y el clima, contribuyendo de esta manera a inferir su comportamiento ante futuros cambios ambientales.

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Objetivos

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2 OBJETIVOS DE LA TESIS DOCTORAL

El objetivo principal de esta Tesis Doctoral es valorar el potencial dendroecológico de los elementos conductores primaverales del roble (*Quercus robur* L.) y del rebollo (*Quercus pyrenaica* Willd.) mediante su empleo para la comprensión de los principales factores ambientales que controlan el crecimiento en la transición entre las regiones biogeográficas atlántica y mediterránea en el noroeste de la Península Ibérica.

Específicamente, los objetivos que se plantean son los siguientes:

- Determinar cuáles son los parámetros anatómicos idóneos para la identificación de las respuestas adaptativas de la formación de madera a las condiciones ambientales, así como su optimización para el estudio de las mismas.
- Identificar las principales variables climáticas con influencia relevante sobre el crecimiento radial, así como su variación espacio-temporal e interespecífica.
- Evaluar la estabilidad temporal de la señal climática en los parámetros xilemáticos, así como su repercusión sobre el potencial uso como *proxies* de los acontecimientos ocurridos durante el pasado.
- Establecer una metodología eficiente para la elaboración de series temporales a partir de elementos anatómicos de la madera temprana en especies de anillo poroso.

La parte fundamental de los ***Resultados*** de la presente Tesis Doctoral está integrada por cinco capítulos con formato de artículo de investigación redactados en inglés, cuyo contenido se adapta a los requisitos para su envío a las correspondientes revistas de ámbito científico. Estos artículos han sido concebidos de manera independiente, de modo que sus contenidos han sido discutidos a partir de la bibliografía existente; las relaciones entre los mismos han sido abordados en la ***Discusión General***.

En dichos artículos se explican las técnicas y análisis aplicados en cada caso particular, así como los aspectos más relevantes de cada área de estudio. No obstante, toda esta información aparece detallada más exhaustivamente en el capítulo general de ***Material y Métodos***, donde puede ser consultada en caso necesario. Asimismo, el capítulo general de Resultados se inicia con un resumen de cada uno de estos artículos, siguiendo el esquema de esta Tesis Doctoral.

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Material y Métodos

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3 MATERIAL Y MÉTODOS

3.1 CARACTERIZACIÓN DE LAS ÁREAS DE ESTUDIO Y LOCALIDADES MUESTREADAS

En la presente Tesis se estudiaron 13 localidades con masas de roble y rebollo ubicadas en las regiones biogeográficas Eurosiberiana y Mediterránea en el noroeste de España. De éstas, 11 se ubican en diferentes zonas de la Comunidad Autónoma de Galicia, mientras que las dos restantes se sitúan en la Comarca de Sanabria, en el norte de la provincia de Zamora, Comunidad Autónoma de Castilla y León, concretamente en el Parque Natural del Lago de Sanabria y Alrededores (Figura 2).

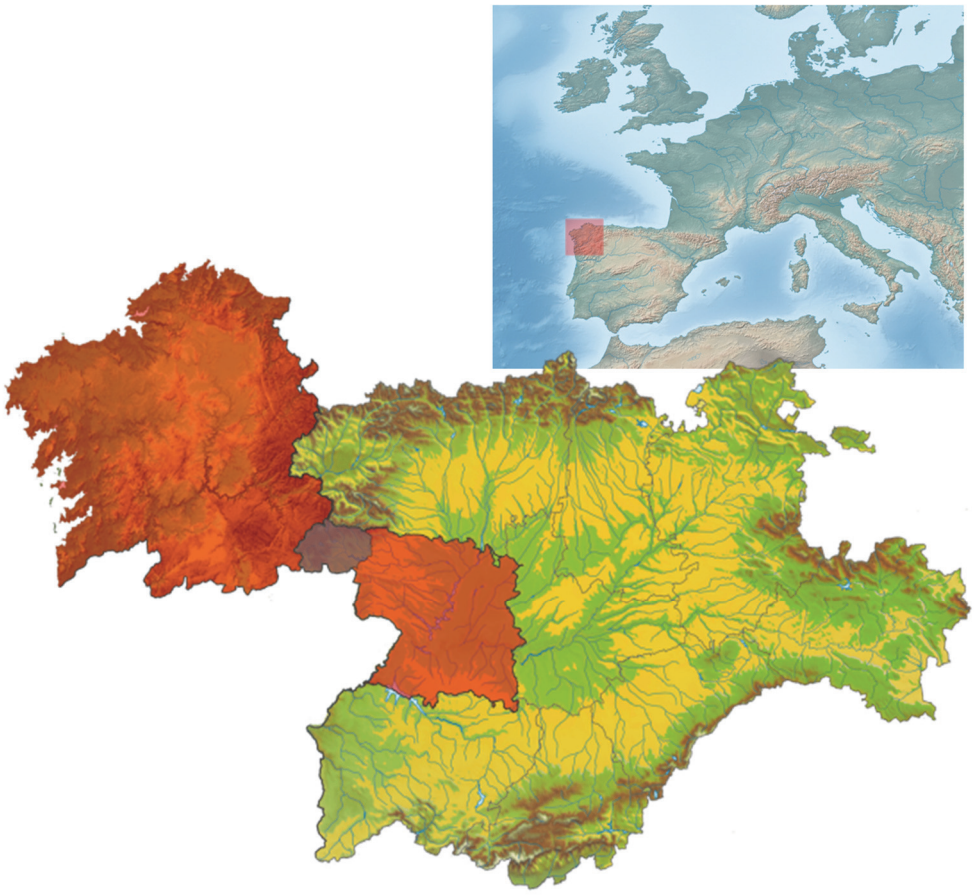


Figura 2. Mapa de situación. (Fuente: Elaboración propia a partir de cartografía del SITGA y del SIGMENA).

Se seleccionaron masas de *Quercus robur* y *Q. pyrenaica* que tuvieran una extensión suficiente y un número elevado de individuos maduros que proporcionasen series dendrocronológicas suficientemente largas para evaluar el comportamiento de las variables relacionadas con los elementos conductores del xilema a lo largo de un período de varias décadas. Asimismo, se seleccionaron bosques en los que no existiera riesgo de hibridación entre ambas especies, de forma que la presencia de individuos híbridos no interfiriese en los resultados (Tabla 1). Para ello se realizaron una serie de observaciones fenológicas previas en numerosos puntos de la geografía gallega y durante dos estaciones de crecimiento completas, seleccionando posteriormente los puntos de muestreo en aquellas zonas en las que el desfase de brotación y floración entre ambas especies impidiese la hibridación, al menos en un alto porcentaje.

Localidad	Código	Especie	Superficie (ha)	Altitud (m)	Orientación	Pendiente (%)	Coord. Geográficas
Fraga dos Casás	**MER**	*Quercus robur*	100	150	Norte	>50	43,59 ºN; 7,91 ºO
Fraga de Vilapena	**VIP**	*Quercus robur*	140	550	Norte	35-40	43,41 ºN; 7,22 ºO
Estornín	**EST**	*Quercus robur*	65	815	Este	15-30	43,05 ºN; 7,22 ºO
Cabanavella	**CAB**	*Quercus robur*	435	1200	Sur-Suroeste	>50	42,80 ºN; 6,89 ºO
Ramirás	**RAM**	*Quercus robur*	1,2	450	Norte	5-10	42,18 ºN; 8,00 ºO
Vilanova dos Infantes	**INF**	*Quercus robur*	2,7	480	Sur	15-30	42,17 ºN; 7,96 ºO
Villaoscura	**VIO**	*Quercus pyrenaica*	4	335	Sur	0-5	42,47 ºN; 7,61 ºO
Sober	**SOB**	*Quercus pyrenaica*	4,5	473	Sur	0-5	42,44 ºN; 7,59 ºO
Castro Caldelas	**CAL**	*Quercus pyrenaica*	130	620	Noreste	>50	42,40 ºN; 7,42 ºO
Fradelo	**FRA**	*Quercus pyrenaica*	43	710	Este	15-30	42,17 ºN; 7,13 ºO
A Gudiña	**GUD**	*Quercus pyrenaica*	36	950	Norte	40-50	42,04 ºN; 7,13 ºO
Vegas de Galende	**GAL**	*Quercus pyrenaica*	112	1000	Suroeste	< 5-15	42,11 ºN; 6,69 ºO
Monte Devesa	**RIB**	*Quercus pyrenaica*	80	1300	Norte	35-40	42,11 ºN; 6,74 ºO

Tabla 1. Caracterización fisiográfica de las localidades muestreadas.

3.1.1 Comunidad Autónoma de Galicia

Galicia se localiza geográficamente entre los 42º y los 44º de latitud norte y entre los 6,5º y los 9,5º de longitud oeste, en el suroeste de Europa. Limita al norte con el mar Cantábrico, al oeste con el océano Atlántico, al este con las comunidades autónomas del Principado de Asturias y Castilla y León (León y Zamora) y al sur con Portugal. Tiene una superficie de 29.365 km^2 donde se pueden diferenciar dos ámbitos geográficos muy distintos: la costa, mucho más poblada, y el interior, con una densidad demográfica menor de poblamiento disperso y carácter montañoso. También se puede distinguir entre una Galicia septentrional, de carácter claramente atlántico, y una Galicia meridional, con un carácter más mediterráneo. La diversidad de relieve, clima y vegetación da a Galicia un carácter bien diferenciado, tanto en el litoral, que se resuelve en una costa articulada en rías, como en el interior montañoso (Bosque Maurel & Vila Valenti 1992).

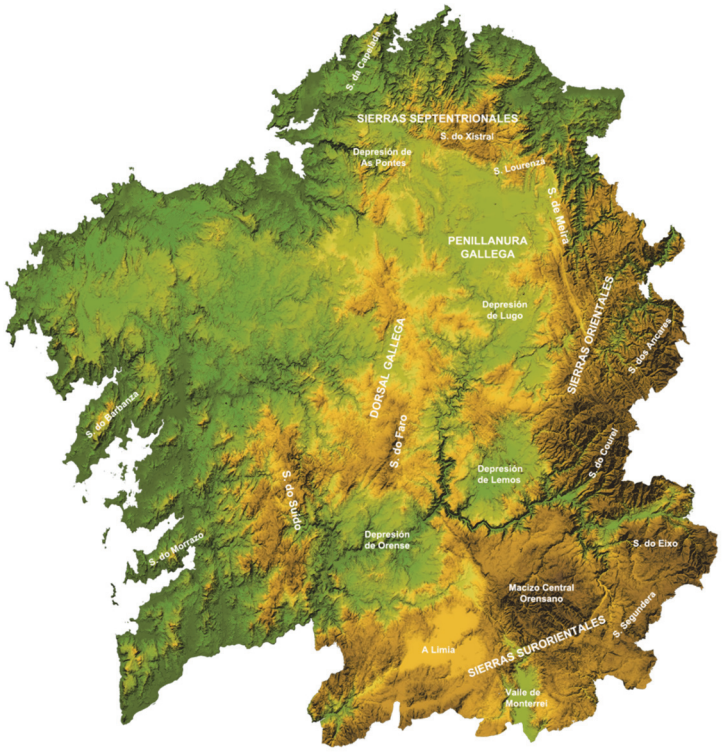


Figura 3. Mapa del relieve de Galicia (Fuente: Elaboración propia a partir de cartografía del SITGA).

El relieve se desarrolla sobre un macizo antiguo, consistente en la base granítica de una antigua cordillera que hoy está casi totalmente erosionada. No obstante, este conjunto fue abombado durante la orogenia alpina, por lo que actualmente las altitudes van desde el nivel del mar hasta los 1.600-2.100 metros de altitud, que se alcanzan en las más altas cumbres de las montañas. La mayor parte de Galicia se encuentra a una altitud de 500-600 metros sobre el nivel del mar. Existe una cadena de sierras prelitorales en dirección norte-sur, la llamada Dorsal Gallega (Figura 3), discontinua y con altitudes máximas de unos 1.000 metros, que separa la Terra Chá, la meseta de Lugo y el valle del Miño de la costa. Las mayores altitudes se encuentran en Peña Trevinca (2.095 m) y Cabeza de Manzaneda (1.778 m) en las Sierras Orientales. En este conjunto se desarrolla un típico relieve fracturado, con múltiples fallas y bloques elevados y hundidos. Los bloques elevados forman las sierras graníticas y los bloques hundidos

cubetas rellenas de sedimentos cuaternarios, con suelos bien desarrollados apropiados para la agricultura (Vidal Romaní 1996).

La comunidad autónoma de Galicia posee un **clima lluvioso**, ya que toda ella recibe la influencia de los vientos dominantes del oeste que traen masas de aire húmedas, ya sean éstas de origen polar o tropical. No obstante, la frecuencia y distribución de las lluvias no son en absoluto las mismas en toda la región. En el norte existe un clima netamente marítimo mientras que en el sur existe un clima con tendencia a la mediterraneidad (Martínez Cortizas et al. 1999). Por su posición geográfica, Galicia posee temperaturas suaves, con una amplitud térmica reducida y unas precipitaciones abundantes, casi siempre por encima de los 800 mm, cuyo máximo se alcanza en invierno. En verano puede haber uno o dos meses de sequía, lo que pone de manifiesto la influencia del clima mediterráneo. No obstante, existen grandes diferencias locales en el régimen de temperaturas a causa del efecto topográfico de las cadenas montañosas. En las sierras prelitorales que superan los 1.000 metros y el Macizo Galaico-Leonés, las temperaturas son frías en invierno y frescas en verano, con uno o dos meses de heladas seguras y de precipitaciones en forma de nieve. Las zonas costeras están sometidas a vientos constantes, que frecuentemente llegan a ser fuertes, pero la presencia de nieve es muy improbable o en todo caso, ocasional. La zona de la meseta de Lugo y Terra Chá es ligeramente más seca que la costa, con cierta tendencia a la continentalidad debido a la presencia de las sierras prelitorales. En las depresiones orensanas es frecuente que se den situaciones de inversión térmica que provocan nieblas persistentes en el fondo de los valles. En las montañas interiores son frecuentes las lluvias orográficas, provocadas por los vientos oceánicos que empujan las masas de aire húmedo sobre relieves que superan los 1.500 metros de altitud.

El suelo depende fundamentalmente de la naturaleza de la roca madre, que en el caso de Galicia es mayoritariamente granito (Barral Silva & Díaz-Fierros Viqueira 1996). El predominio de substratos litológicos silíceos ocasiona que los suelos mayoritarios sean pobres en nutrientes y con pH ácido, siendo más aptos para la agricultura los localizados en las depresiones, vegas y vaguadas debido a la acumulación de sedimentos cuaternarios, mucho más ricos. Éstos, junto con los escasos afloramientos de rocas carbonatadas del sector más oriental de Galicia y las áreas de roquedo básico-ultrabásico de los complejos ofiolíticos de Capelada, Sobrado-Melide, Monte Castelo y Santiago-Lalín-Forcarei, son los únicos ejemplos de suelos básicos de la Comunidad (Figura 4).

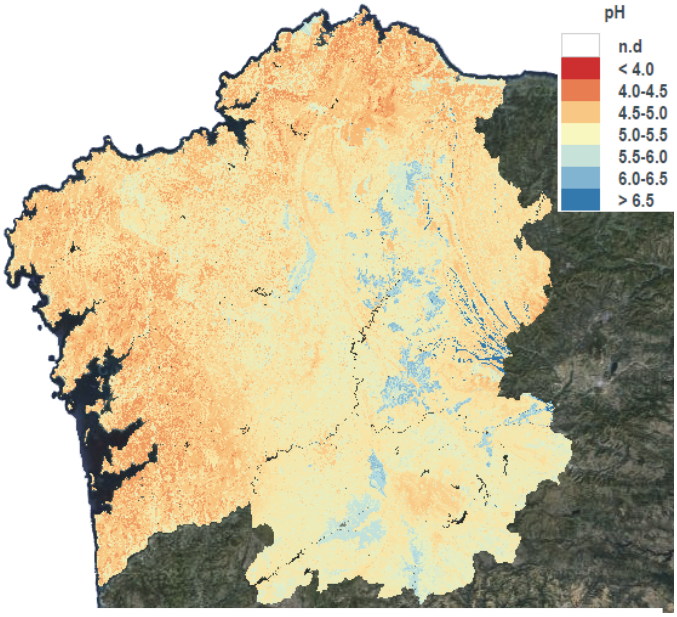


Figura 4. Mapa de pH de los suelos de Galicia (Fuente: http://rgis.cesga.es/solos/solos.html) .

Como consecuencia, **la vegetación** gallega se compone sobre todo de especies silicícolas o acidófilas capaces de crecer sobre suelos pobres, habiendo sido intensamente transformada a lo largo de la historia por la acción antrópica. La actuación más destacada y que más ha alterado el paisaje rural han sido las repoblaciones forestales, sobre todo a partir de 1950, con especies de crecimiento rápido y rentables desde el punto de vista económico, especialmente pinos (*Pinus pinaster* Ait., *P. radiata* D. Don) y eucaliptos (*Eucalyptus globulus* Labill., *E. nitens* H.Deane & Maiden). La **vegetación climácica** está dominada por los robles (Figura 5), especialmente el melojo o rebollo (*Quercus pyrenaica* Willd.), más abundante en el interior y el sur, y el carballo (*Quercus robur* L.) en el resto del territorio, formando el tipo de bosque mayoritario del piso basal (Izco 1996). En el piso montano aparece el haya (*Fagus sylvatica* L.) excepcionalmente, relegada a las sierras más húmedas del interior. Más abundante en este piso es el

avellano (*Corylus avellana* L.) y las especies subseriales como acebo (*Ilex aquifolium* L.), tejo (*Taxus baccata* L.) y arce (*Acer pseudoplatanus* L.). En el piso montano superior predomina el abedul (*Betula alba* L.), mezclado con serbales (*Sorbus aucuparia* L.), aunque los abedules son frecuentes a cualquier altitud conviviendo con acebos o avellanos, y en los suelos más hidromorfos con alisos (*Alnus glutinosa* (L.) Gaertn), sauces (*Salix atrocinerea* Brot., *S. caprea* L., *S. viminalis* L., *S. eleagnos* Scop., etc.), fresnos (*Fraxinus excelsior* L.) y olmos (*Ulmus glabra* Huds.). En los valles orientados hacia la meseta del sur orensano, aparecen especies mediterráneas como la encina (*Quercus ilex* subsp. *ballota*). También tiene mucha importancia el alcornoque (*Quercus suber* L.) en las áreas de mayor influencia mediterránea del sur de Galicia, tanto en el interior como en la costa, pero siempre a baja altitud. Sin embargo, más de la cuarta parte de la superficie de la región está ocupada por matorrales, etapas seriales de degradación del bosque, por causas naturales pero especialmente antrópicas, sobre todo debido a la deforestación secular y a los frecuentes incendios.

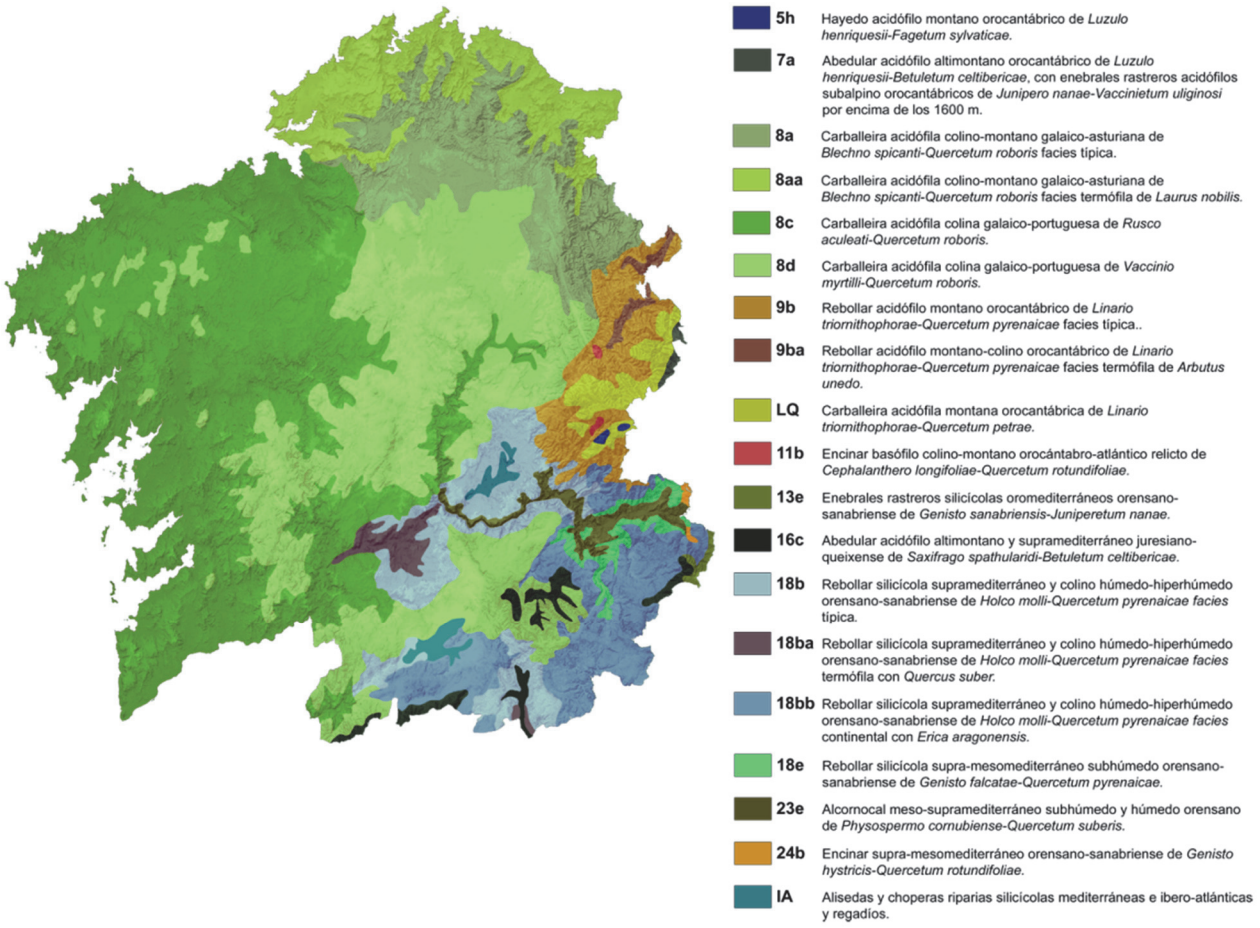


Figura 5. Mapa de vegetación potencial de Galicia (Fuente: Elaboración propia basado en Rivas-Martínez (1987)).

Las formaciones de matorrales que predominan en estos ambientes alterados o degradados son el tojo (*Ulex europaeus* L.) y los brezos (*Erica australis* L., *E. scoparia* L.) en las zonas más secas, mientras que los piornos (*Cytisus multiflorus* (L´Hér.) Sweet), las jaras (*Cistus ladanifer.* L.) y los enebros (*Juniperus communis* subsp. *nana*) lo hacen en las zonas más elevadas, aunque éstos últimos frecuentemente como comunidades climácicas de alta montaña.

Para facilitar la descripción de cada una de las 11 localidades estudiadas en Galicia se han distinguido cuatro grandes unidades descriptivas, en función de los diferentes dominios climáticos y de relieve que existen en la región (Figura 6).

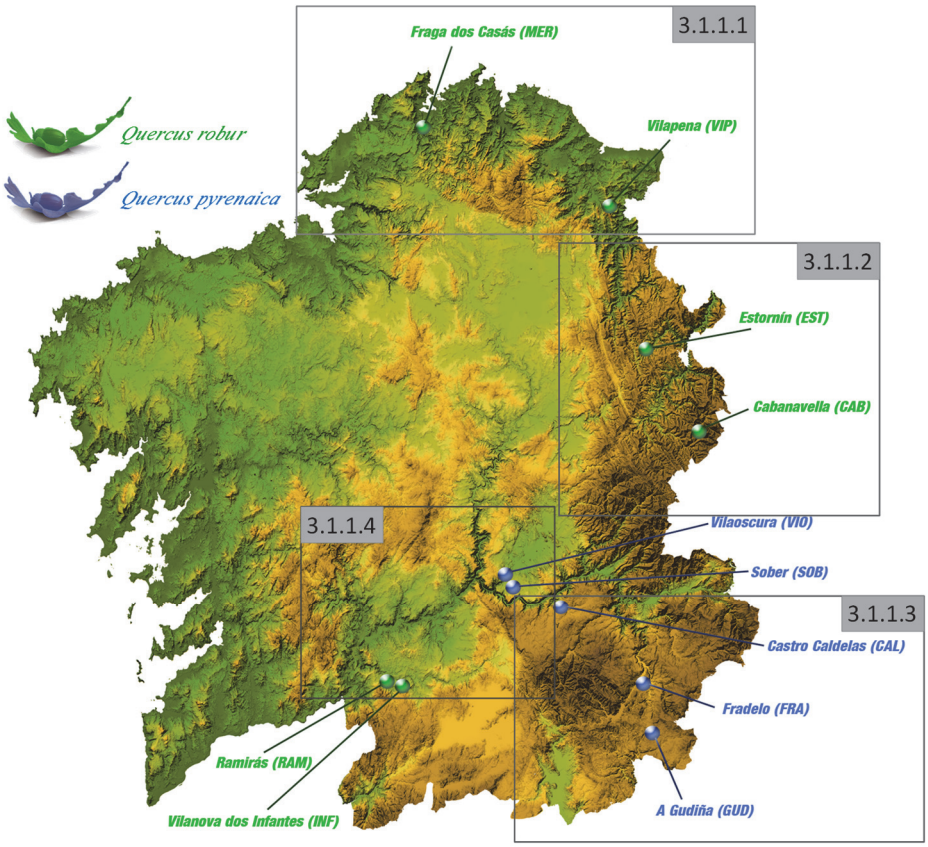


Figura 6. Localidades muestreadas en Galicia y unidades geográficas descriptivas.

3.1.1.1 La Galicia Cantábrica.

3.1.1.1.1 Localización geográfica

La Galicia cantábrica se extiende por toda la franja septentrional de la provincia de Lugo y por la península del cabo Ortegal, ya en la provincia de A Coruña. Abarca el territorio comprendido entre las rías abiertas a lo largo de este litoral y la alineación de sierras que discurre por el norte de Galicia (Xistral, Gañidoira, Faladoira, Coriscada, Capelada, Meira, Pousadoiro y Lourenzá), incluyendo una serie de depresiones como las de Mondoñedo y O Valadouro. Desde el punto de vista de la división administrativa incluye una veintena de municipios que abarcan en conjunto una extensión próxima a los 2.000 km^2 (Rodríguez Lestegás 1996). En la vertiente cantábrica se estudiaron dos localidades, la **Fraga dos Casás**, situada en la cuenca del río Mera, por detrás de la ría de Santa Marta de Ortigueira, provincia de A Coruña, y la **Fraga de Vilapena**, en la sierra da Cadeira perteneciente al municipio de Trabada, provincia de Lugo.

3.1.1.1.2 Materiales litológicos y suelos

Los materiales más abundantes que aparecen son pizarras, esquistos, areniscas y cuarcitas (Figura 7), con presencia de pizarras negras de la serie de Luarca en la sierra da Faladoira. Dentro de la zona

destacan las unidades Cedeira y Capelada, donde predominan los gneises, las anfibolitas, las peridotitas y las eclogitas, y la unidad de Moeche, constituida por abundantes rocas verdes (pizarras con intercalación de serpentinitas, anfibolitas, etc.) (Bastida et al. 1984).

Los suelos formados en equilibrio con las condiciones ambientales de la Galicia Cantábrica son, según la clasificación de la FAO (2007), Cambisoles húmicos o dístricos, desarrollados sobre esquistos, granitos y rocas básicas (Macías 1986). En la Fraga dos Casás, los suelos son de tipo Cambisol desarrollados sobre rocas básicas (Metaperidotitas), que disminuyen de espesor en las partes altas y de fuerte pendiente por causa de la erosión, pasando a ser suelos esqueléticos del tipo Umbrisol dístrico. Por otra parte, los suelos encontrados en la Fraga de Vilapena son suelos desarrollados a partir de pizarras y areniscas de tipo Leptosol úmbrico y Cambisol muy orgánico que se hace coluvial en los lugares de fuerte pendiente. En estos casos origina verdaderos Podsoles, aunque no muy desarrollados(Guitian Ojea 1974).

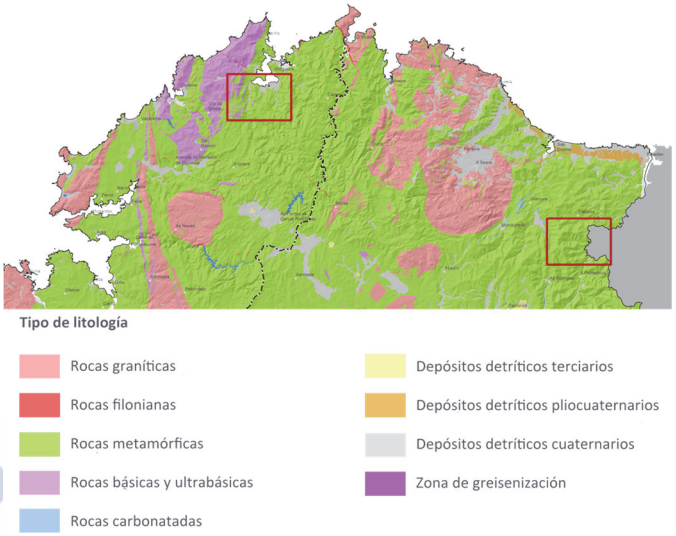
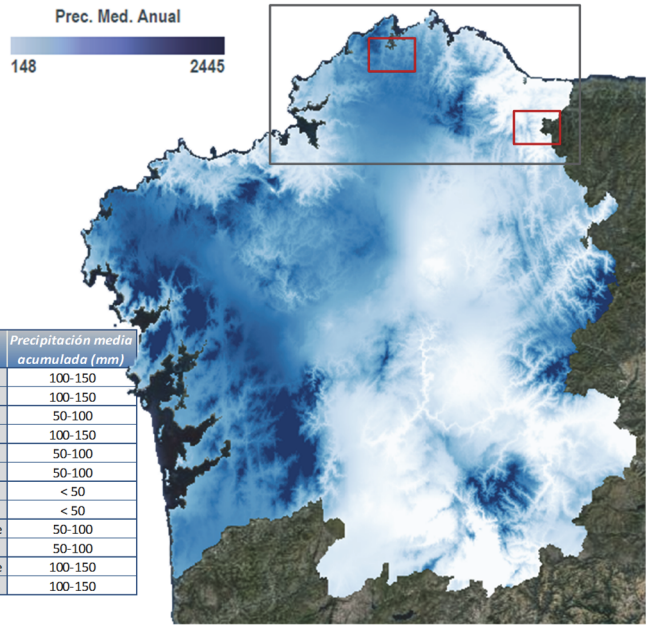


Figura 7. Mapa litológico de la franja cantábrica gallega, con la localización de la Fraga dos Casás (izqda.) y de la Fraga de Vilapena (dcha.) (Fuente: Elaboración propia a partir de cartografía del IGME).

3.1.1.1.3 Caracterización climática

La zona cantábrica gallega posee unas condiciones climáticas en las que la suavidad térmica y la elevada pluviosidad son las notas dominantes. El hecho de que se trate de un área litoral le confiere una moderación térmica impuesta por la proximidad del mar y, en este caso, reforzada por los vientos templados del oeste y por la presencia del flujo oceánico cálido de la Corriente del Golfo. Además existen una serie de modificaciones climáticas impuestas por el relieve, sobre todo en el dominio de las sierras septentrionales gallegas, con altitudes por encima de los 1.000 m. En estas circunstancias, el aire ártico llega en contadas ocasiones a través de una circulación meridiana que traslada esencialmente aire marítimo, dejando heladas en el litoral y rigurosos fríos en las áreas interiores, sobre todo en los núcleos montañosos; las nevadas son excepcionales. Más frecuentes son las masas de aire polar frescas y húmedas de carácter marítimo que llegan a este territorio durante el invierno. Por el contrario, durante el verano son las masas de aire tropical marítimo las que determinan la estabilización atmosférica (Martínez Cortizas et al. 1999).

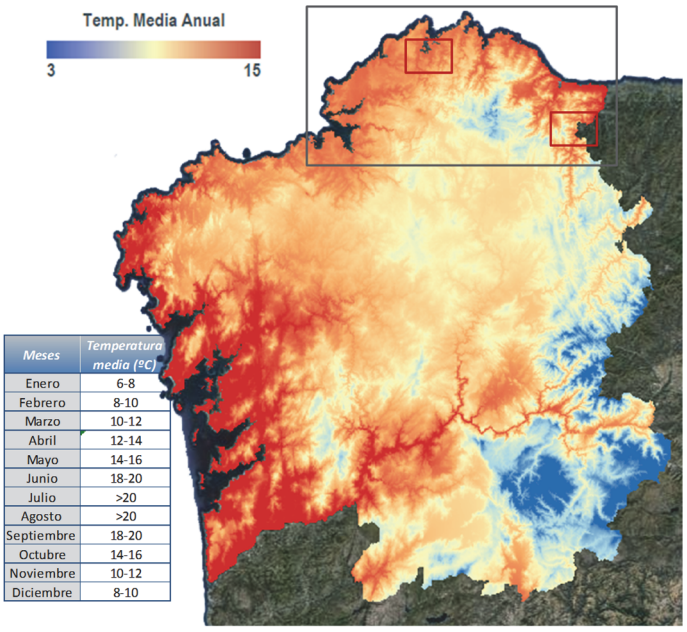


Meses	Precipitación media acumulada (mm)
Enero	100-150
Febrero	100-150
Marzo	50-100
Abril	100-150
Mayo	50-100
Junio	50-100
Julio	< 50
Agosto	< 50
Septiembre	50-100
Octubre	50-100
Noviembre	100-150
Diciembre	100-150

Figura 8. Precipitación media anual (mm) y tabla de rangos de precipitación media mensual acumulada para la Fraga dos Casás (izqda.) y para la Fraga de Vilapena (dcha.) (Fuente: http://rgis.cesga.es/solos/solos.html).

Las precipitaciones alcanzan valores de entre 1.000 y 1.500 mm anuales. En esta zona de Galicia prácticamente todos los meses del año entran en la categoría de lluviosos, no existiendo un período seco como tal. No obstante, la mayor concentración de lluvias se produce en el invierno (noviembre-marzo), en el que se recoge alrededor del 60% del valor anual. Durante los meses estivales (junio-septiembre) es cuando se da el menor registro, de aproximadamente el 20% del total anual. El gradiente pluviométrico altitudinal calculado a partir del Atlas Climático (Martínez Cortizas et al. 1999) es de 106 mm/100 m para la Fraga dos Casás y de 100 mm/100 m para la Fraga de Vilapena. El régimen pluviométrico mantiene un desarrollo muy constante y con cifras bastante elevadas durante todo el año, a excepción de la época estival, en la que se acusa una relativa disminución de las lluvias en relación al resto de las estaciones, que presentan casi idéntica frecuencia de precipitaciones (Figura 8). Son muy habituales las nieblas, con una carga de humedad tan fuerte que pueden causar la saturación total del ambiente (Rodríguez Lestegás 1996).

Las temperaturas medias anuales oscilan entre 12 y 14 ºC, lo que se puede considerar normal para este sector de la cornisa cantábrica. Las temperaturas medias más bajas corresponden a los meses de diciembre, enero y febrero, rondando siempre los 10 ºC salvo en las zonas de montaña, donde pueden registrarse hasta 5 ºC. El gradiente termométrico calculado a partir del Atlas Climático (Martínez Cortizas et al. 1999) es de –0,36 ºC/100 m para la Fraga dos Casás y de -0,67 ºC/100 m para la Fraga de Vilapena. Por tanto, las localidades de estudio presentan una estación invernal corta y muy benigna debido a la acción moderadora del océano. Las temperaturas medias más altas se registran durante los meses de julio y agosto (Figura 9), con valores que no suelen superar los 18 ºC, por lo que se puede decir que los veranos tienen una duración normal pero son muy poco calurosos. De esta suavidad térmica deriva una restringida amplitud térmica anual, con valores que rondan los 9-11 ºC (Rodríguez Lestegás 1996).



Meses	Temperatura media (ºC)
Enero	6-8
Febrero	8-10
Marzo	10-12
Abril	12-14
Mayo	14-16
Junio	18-20
Julio	>20
Agosto	>20
Septiembre	18-20
Octubre	14-16
Noviembre	10-12
Diciembre	8-10

Figura 9. Temperatura media anual (ºC) y tabla de rangos de temperatura media mensual para la Fraga dos Casás (izqda.) y para la Fraga de Vilapena (dcha.) (Fuente: http://rgis.cesga.es/solos/solos.html).

3.1.1.1.4 Vegetación

Corológicamente, la Galicia cantábrica pertenece a la Región Eurosiberiana, Provincia Cántabro-Atlántica (Subprovincia Astur-Galaica), Sector Galaico-Asturiano, Subsector Galaico Septentrional (Rivas-Martínez 1987).

En esta zona, la vegetación climácica se corresponde con una **Carballeira acidófila colino-montana galaico-asturiana o *Blechno spicanti-Quercetum roboris*** (Figura 10), que abarca altitudinalmente desde el nivel del mar hasta los 1.000 m de altitud. Se desarrolla sobre granitos, pizarras, esquistos, cuarcitas, gneises o rocas ultrabásicas, que originan suelos profundos y desarrollados de tipo tierra parda, a veces podsolizada, de estructura arcillosa y con humus evolucionado. El pH y la concentración de nutrientes son bajos, pero la presencia de arcillas y el alto contenido en materia orgánica permiten que haya un elevado grado de humedad edáfica. Estas formaciones se sitúan en laderas de pendiente media, presentando una estructura en tres estratos: el superior lo integran árboles

de 15-20 m de altura, en el que domina el carballo (*Quercus robur*) acompañado por castaños (*Castanea sativa* Miller), abedules (*Betula alba*) y arces (*Acer pseudoplatanus*) más raramente, con una cobertura del 50-75%. El estrato intermedio lo ocupan arbustos y árboles bajos de 1-3 m y baja cobertura (10-30%), siendo muy frecuentes los avellanos (*Corylus avellana*) acompañados de otras especies como acebos (*Ilex aquifolium*), arraclán (*Frangula alnus* Miller), retamas (*Cytisus scoparius* (L) Link.), carqueixas (*Pterospartum tridentatum* L.) y distintas especies de brezos (*Erica sp.)*. El estrato inferior está constituido por un tapiz herbáceo de cobertura media y altura de 10-60 cm en el que dominan el arándano (*Vaccinium myrtillus* L.) y diversas herbáceas, siendo frecuentes los tapices de musgos y líquenes.

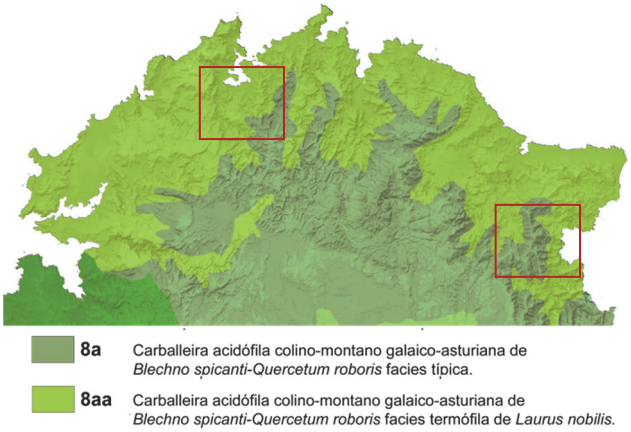


Figura 10. Vegetación potencial de la franja cantábrica gallega, con la localización de las dos fragas estudiadas (Fuente: Elaboración propia basado en Rivas-Martínez (1987)).

La Fraga de Vilapena corresponde a la facies típica de esta formación (Figura 10, dcha.), mientras que la Fraga dos Casás corresponde a la facies termófila de *Laurus nobilis* (Figura 10, izqda.). Esta variante, en la que se incorporan al bosque el laurel y el madroño (*Arbutus unedo* L.), así como algunos helechos termófilos mararonésicos como *Culcita macrocarpa* C. Presl., *Davallia canariensis* (L.) Sm. o *Woodwardia radicans* (L.) Sm, aparece en las zonas de mayor termicidad, generalemte a altitudes inferiores a los 250 m (Silva Pando & Rigueiro Rodríguez 1992).

El sotobosque es una formación denominada genéricamente landa, en buena parte de condición subserial, pero ocasionalmente de carácter climácico, allí donde la instalación del bosque es limitada. Se trata de un matorral formado por especies como la zarza (*Rubus sp.*), el tojo (*Ulex sp.*), la retama (*Cytisus scoparius*), la carqueixa (*Pterospartum tridentatum*) y distintas especies de brezo (*Erica arbórea* L., *E. cinérea* L., *E. umbellata* L., *E. ciliaris* L., *E. mackaiana* L., *Calluna vulgaris* (L.) Hull, *Daboecia cantábrica* L.). A este estrato arbustivo hay que añadirle otro de carácter herbáceo y también numerosos helechos (*Blechnum spicant* (L.) Sm., *Dryopteris aemula* (Aiton) Kuntze, *D. dilatata* (Hoffm.) A.Gray, *Polystichum setiferum* (Forssk.) Woynar., *Pteridium aquilinum* L.) y líquenes.

3.1.1.1.5 Localidades muestreadas

En las dos localidades muestreadas en la Galicia Cantábrica domina la especie *Quercus robur*. En la cuenca del río Mera, y dentro del extenso bosque denominado Fraga dos Casás, se seleccionó una de las laderas que flanquean dicho río, con una elevada pendiente (MER). En la Mariña Oriental Lucense, en el entorno conocido como Fraga de Vilapena, se seleccionó una masa cercana a la población del mismo nombre (VIP).

Fraga dos Casás (MER)

La Fraga dos Casás está incluida en el LIC Ortigueira-Mera, ocupa una superficie de aproximadamente 100 ha y está situada a una altitud de unos 150 m (Figura 11).

En el estrato arbóreo domina *Quercus robur* en la totalidad de su extensión, con algunos rodales de *Quercus pyrenaica* únicamente en la parte alta de la fraga. Como vegetación acompañante aparecen frecuentemente pies de *Laurus nobilis* L. y de *Arbutus unedo* L, éstos últimos de bastante tamaño. En una de las vaguadas de la fraga, el madroño es la especie dominante y son los robles los que aparecen en menor número. Hacia la parte baja, ligados a los cursos de agua, aparecen numerosos pies de *Woodwardia radicans*(L.) Sm. El sotobosque está compuesto por *Hedera hibernica*, numerosas especies de briófitos,

Rubia peregrina cf. subs. *longifolia*, *Vaccinium mirtyllus*, *Ruscus aculeatus* L., y *Primula vulgaris* L. También es frecuente *Ilex aquifolium* y varias especies de helechos (*Davallia canariensis, Blechnum spicant*, *Polypodium interjectum* Shivas, *Dryopteris affinis*). La fraga se encuentra flanqueada totalmente por repoblaciones de eucalipto (*Eucalyptus globulus*).

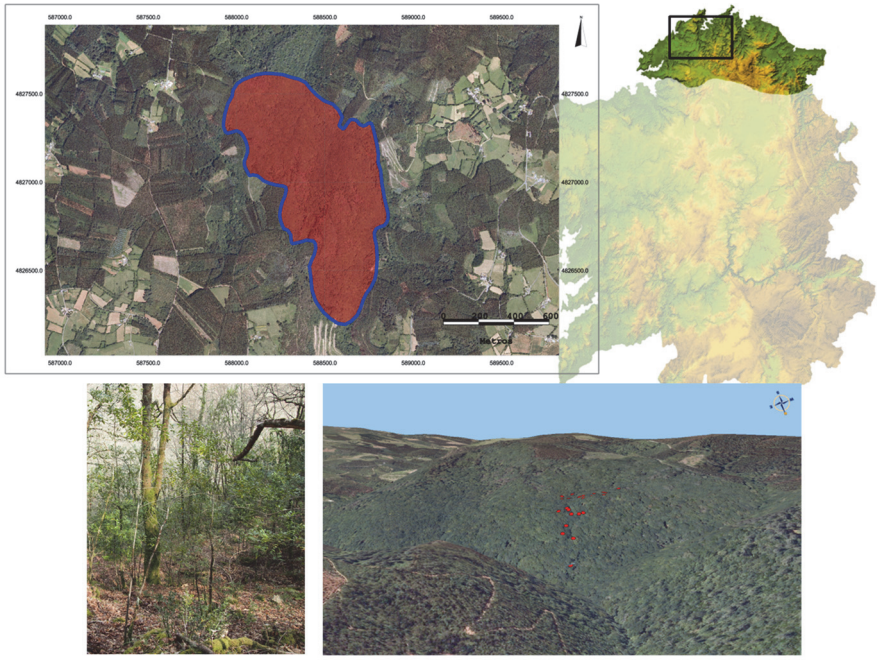


Figura 11. Fraga dos Casás. En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados en color rojo.

Fraga de Vilapena (VIP)

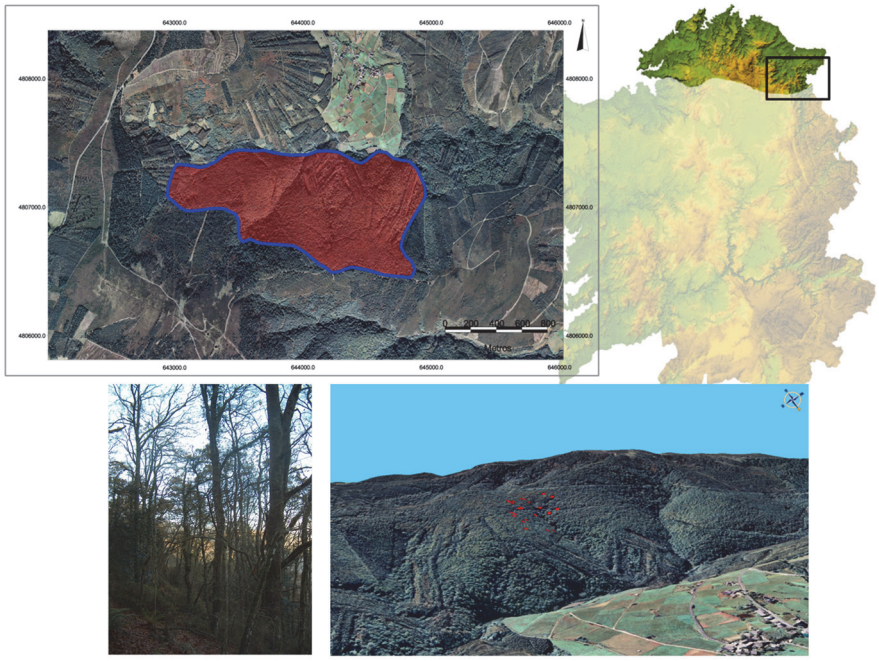


Figura 12. Fraga de Vilapena. En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados en color rojo

La Fraga de Vilapena, cercana a la población del mismo nombre, se encuentra próxima a una morrena terminal de origen glaciar formada en el periodo cuaternario. Se trata de una extensión de 140 ha poblada por roble (*Quercus robur*) y castaño (*Castanea sativa*) en el estrato dominante, apareciendo como vegetación acompañante sauces (*Salix sp.*), avellanos (*Corylus avellana*) y abedules (*Betula alba*).

En la parte baja aparecen alisos (*Alnus glutinosa*) y chopos (*Populus nigra*), habiendo en toda la extensión una gran abundancia de helechos como *Blechnum spicant* o *Dryopteris filix-mas* (L.) Schott (Figura 12). Los árboles muestreados se encuentran en la ladera de umbría, a unos 550 m de altitud y en una zona de marcada pendiente, sobre un sustrato de pizarra. El suelo que predomina en este entorno es de tipo arcilloso húmedo.

3.1.1.2 Sierras Orientales Gallegas

3.1.1.2.1 Localización geográfica

Estas montañas marcan la frontera oriental de Galicia, que la separa de Asturias y León. Se extienden a lo largo de 1.783 km² (Guitián 1985), y en ellas aparecen un conjunto de municipios lugueses de carácter montañés, caracterizados por su gran extensión pero escasa población en comparación con lo que es habitual en Galicia. En las montañas orientales se estudiaron dos localidades: una llamada **Estornín**, perteneciente administrativamente al municipio de Baleira (Fonsagrada), con altitudes medias por encima de los 600 m; y otra llamada **Cabanavella**, perteneciente al municipio de Cervantes y dentro del LIC Ancares-Courel, con elevaciones medias por encima de los 1.300 m.

3.1.1.2.2 Materiales litológicos y suelos

Los materiales que afloran en las Montañas Orientales gallegas son muy antiguos, desde precámbricos a silúricos. Existen materiales sedimentarios precámbricos y cámbricos representados por pizarras y areniscas, y afloramientos graníticos puntuales, algunas formaciones cuaternarias ligadas a fenómenos de glaciación, y menos frecuentemente rocas sedimentarias carbonatadas. Son abundantes por todo el territorio los diques de cuarcita, sobre todo de cuarcitas silúricas (Pérez Alberti 1981).

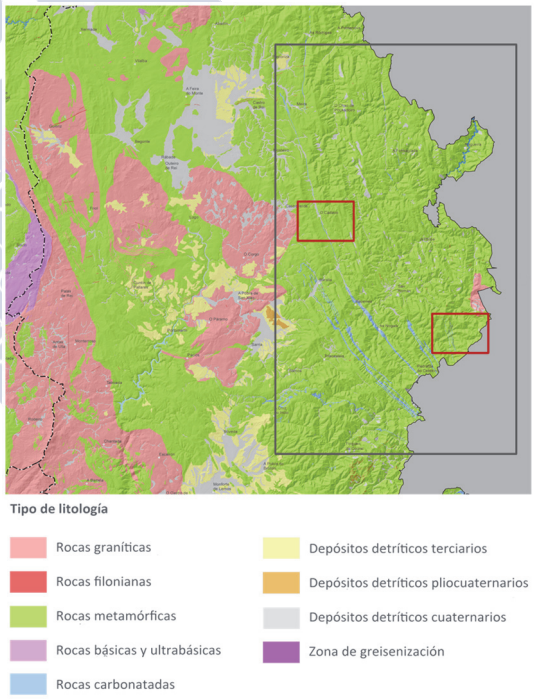


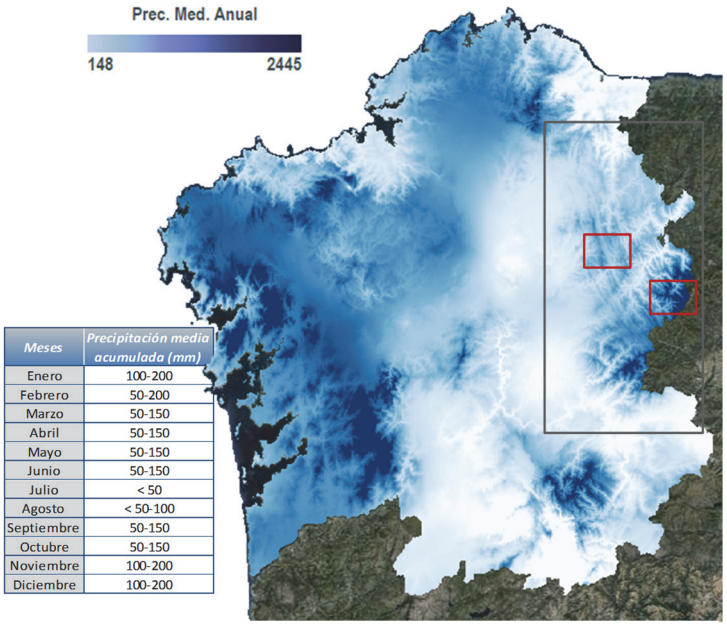
Figura 13. Mapa litológico de las Sierras Orientales, con la localización de Estornín (izqda.) y Cabanavella (dcha.) (Fuente: Elaboración propia a partir de cartografía del IGME).

Los suelos más abundantes en el territorio, y que aparecen en las dos localidades estudiadas, son los formados sobre pizarra y esquisto (Figura 13), ácidos y pobres en nutrientes, pero con una importante cantidad de materia orgánica en el horizonte superior, de color oscuro intenso y alto contenido en arcilla (Guitián 1985). Aparecen suelos de perfil A-R, con epipedon ócrico y profundidad menor de 10 cm (Leptosoles líticos) o con epipedon úmbrico y profundidad menor de 30 cm (Leptosoles úmbricos); sobre la roca fresca o poco alterada. Cuando el grado de desarrollo de los suelos está condicionado por el equilibrio entre los procesos de erosión-sedimentación, los más extendidos son los Regosoles úmbricos, que presentan un horizonte A úmbrico y se forman a partir de materiales no consolidados. Asociados a ellos aparecen, en menor

medida, Cambisoles húmicos, con horizonte B cámbico y epipedon úmbrico (Martínez Cortizas et al. 1993).

3.1.1.2.3 Caracterización climática

Como consecuencia de la altitud y la posición interior de la sierra, que actúa como barrera de los vientos atlánticos, el clima está ligeramente continentalizado, con precipitaciones elevadas de distribución irregular. La nieve suele comenzar a caer a finales de septiembre pero las nevadas empiezan a ser persistentes a partir de mediados de noviembre (Martínez Cortizas et al. 1999). Las precipitaciones tormentosas, generalmente estivales, son considerables y palian la sequía estival que produciría la ausencia de lluvias frontales. También son muy frecuentes las nieblas de valle y las inversiones térmicas. Hay heladas intensas y frecuentes durante buena parte del año (Martínez Cortizas et al. 1999).

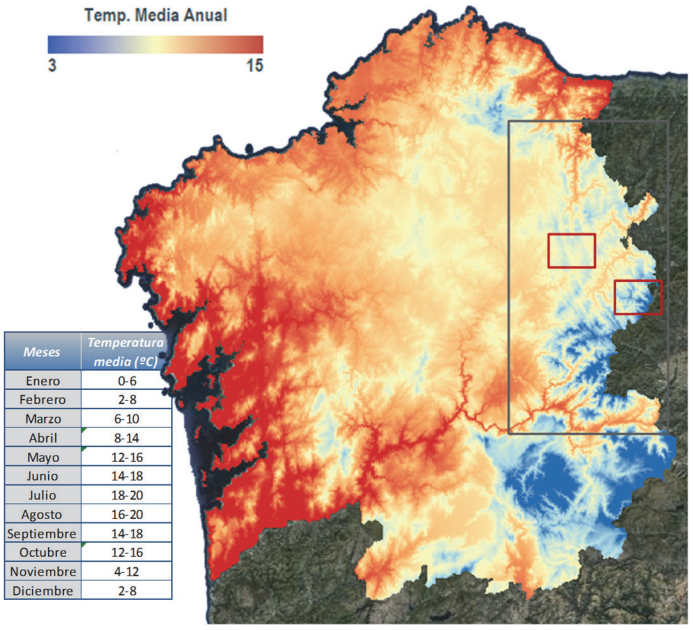


Meses	Precipitación media acumulada (mm)
Enero	100-200
Febrero	50-200
Marzo	50-150
Abril	50-150
Mayo	50-150
Junio	50-150
Julio	< 50
Agosto	< 50-100
Septiembre	50-150
Octubre	50-150
Noviembre	100-200
Diciembre	100-200

Figura 14. Precipitación media anual (mm) y tabla de rangos de precipitación media mensual acumulada para Estornín (dcha.) y Cabanavella (izqda.)
(Fuente: http://rgis.cesga.es/solos/solos.html).

El período de actividad vegetal está restringido a 4-5 meses al año y los fuertes vientos limitan el crecimiento en las cumbres, habiendo sobrealimentación nival en las posiciones a sotavento (Rodríguez Guitián & Guitián Rivera 1993).

Las precipitaciones en general son elevadas, de más de 1.400 mm/año, y tienen carácter atlántico (Martínez Cortizas et al. 1999); en torno a los 1.500 m de altitud la pluviosidad alcanza valores de 2.500 mm (Figura 14), y se acerca a los 2.700 mm anuales en el área subalpina(Rodríguez Guitián & Guitián Rivera 1993). El gradiente pluviométrico altitudinal calculado a partir del Atlas Climático (Martínez Cortizas et al. 1999) es de 75 mm/100 m, y aunque existe un mínimo estival, no se observa ningún mes seco. De hecho, el aporte hídrico estival suele superar los 250 mm a los 1.000 m de altitud (Rodríguez Guitián & Guitián Rivera 1993). Entre otoño e invierno cae alrededor del 70% del total y una buena parte lo hace en forma de nieve, nevando 30 días al año en cotas de 1.100-1.200 m y menos por debajo de esta altitud (Izco 1996).



Meses	Temperatura media (ºC)
Enero	0-6
Febrero	2-8
Marzo	6-10
Abril	8-14
Mayo	12-16
Junio	14-18
Julio	18-20
Agosto	16-20
Septiembre	14-18
Octubre	12-16
Noviembre	4-12
Diciembre	2-8

Figura 15. Temperatura media anual (ºC) y tabla de rangos de temperatura media mensual para para Estornín (dcha.) y Cabanavella (izqda.)
(Fuente: http://rgis.cesga.es/solos/solos.html).

Las temperaturas son frescas durante el verano y frías en el invierno (Figura 15). Es frecuente que haya meses en los que la media de las mínimas quede por debajo de cero, y algunas de las medias mensuales son también negativas, clasificándose el invierno como frío. Existe riesgo de heladas durante muchos meses, incluida la primavera; y todavía se alarga más dicho período con la altura, por lo que por encima de los 1.200 m existe un período libre de heladas de poco más de 100 días al año. A los 1.500 m de altitud los fenómenos de crioturbación abarcan casi nueve meses, y suben a 11 meses en el límite altitudinal de la sierra (Rodríguez Guitián & Guitián Rivera 1993). Las medias más altas se registran en julio y agosto, rondando o superando los 15º C. Las oscilaciones temporales diurnas y estacionales y el contraste de temperaturas son también características del clima de la zona. El gradiente calculado a partir del Atlas Climático (Martínez Cortizas et al. 1999) es de –0,50º C/100 m.

3.1.1.2.4 Vegetación

En las montañas orientales tiene lugar la transición entre las regiones Mediterránea y Eurosiberiana, aunque las dos localizaciones estudiadas en este entorno se encuentran incluidas en la Eurosiberiana, dentro de la Provincia Atlántico-Europea, Subprovincia Orocantábrica, Sector Laciano-Ancarense, Subsector Naviano-Ancarense (Rivas-Martínez 1987).

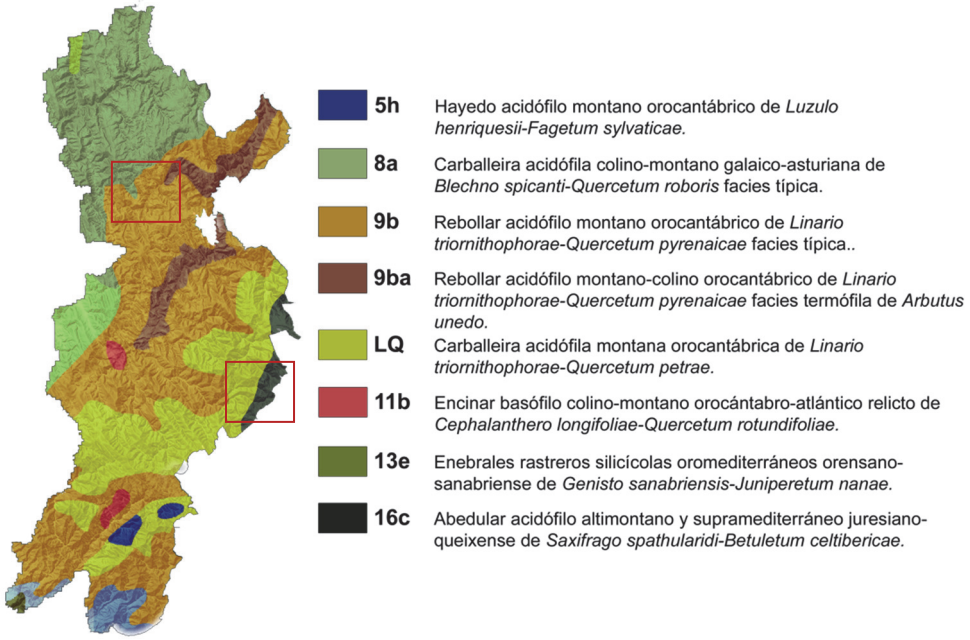


Figura 16. Vegetación potencial de las montañas orientales gallegas, con la localización de Estornín (superior izqda.) y Cabanavella (inferior dcha.) (Fuente: Elaboración propia basado en Rivas-Martínez (1987)).

Por ser una zona de transición, la vegetación climácica en este entorno montañoso es muy rica y variada (Figura 16). En los pisos colino (<600 m) y/o montano (600-1.500 m) aparecen comunidades vegetales como los robledales orocantábricos-galaicos (*Blechno spicanti-Quercetum roboris*), robledales orocantábricos sobre sustratos silíceos (*Linario triornithophorae-Quercetum petraeae*), melojares orocantábricos y astur-galaicos sobre sustratos ácidos (*Linario triornithophorae-Quercetum pyrenaicae*) y abedulares orocantábricos sobre sustratos ácidos (*Luzulo henriquesii-Betuletum celtibericae*), así como distintas asociaciones herbáceas (Silva Pando 1990). Los afloramientos calcáreos permiten, además, la existencia de relictos de vegetación perennifolia, representados por comunidades como los encinares relícticos de talla media/baja (*Genisto falcatae-Quercetum rotundifoliae*), o los hayedos orocantábricos (*Omphalodo nitidae-Fagetum sylvaticae*) (Izco 1996).

Por encima de los 1700 m de desarrollan matorrales climácicos subalpinos, mientras que por debajo de esta altitud, los matorrales constituyen comunidades de carácter serial de estos tipos de bosque, siendo importantes los piornedos, brezales y xesteiras (Géneros *Cytisus, Erica* y *Genista*), que forman asociaciones como *Cytiso scoparii-Genistetum polygaliphyllae, Genistello tridentatae-Ericetum aragonensis, Ulici europaei-Cytisetum striati* subas. *Cytisetosum multiflori o Genisto anglicae-Ericetum tetralicis,* entre otras (Rodríguez Guitián & Guitián Rivera 1993).

Los robledales caducifolios de las asociaciones ***Blechno spicanti-Quercetum roboris***, en la localidad de Estornín y ***Linario triornithophorae-Quercetum petraeae***, en la de Cabanavella, son las formaciones vegetales muestreadas en las sierras orientales gallegas (Figura 16).

El robledal acidófilo colino-montano galaico-asturiano de *Blechno spicanti-Quercetum roboris,* en su facies típica, es un robledal típicamente acidófilo en el que domina sobre todo *Quercus robur* y, en menor medida, *Castanea sativa* (Silva Pando 1990), del mismo tipo que el descrito para la Fraga de Vilapena.

El robledal acidófilo montano orocantábrico de *Linario triornithophorae-Quercetum petraeae* se corresponde con una formación en la que domina *Quercus robur*, *Q. petraea* (Mattuschka) Liebl. y sus híbridos (Vila-Lameiro & Díaz-Maroto Hidalgo 2002), asentada sobre sustratos silíceos, que en numerosas ocasiones representan la vegetación clímax en este territorio. Con relativa frecuencia se presentan también como codominantes las especies *Betula alba* y *Acer pseudoplatanus*, siendo acompañantes *Sorbus aucuparia*, *Sorbus aria* (L.) Crantz, *Corylus avellana* e *Ilex aquifolium*. Se sitúan en exposiciones norte y a veces oeste, en zonas de umbría más acusada y menor sequía estival (Silva Pando 1990).

3.1.1.2.5 Localidades muestreadas

En las sierras orientales gallegas se muestrearon dos masas de *Quercus robur*, una de ellas en el municipio de Baleira, que se denominó Estornín (EST) por encontrarse cerca de la población del mismo nombre; y la otra en los Ancares lucenses, llamada Cabanavella (CAB) por ser el nombre que dan a estas laderas en la zona.

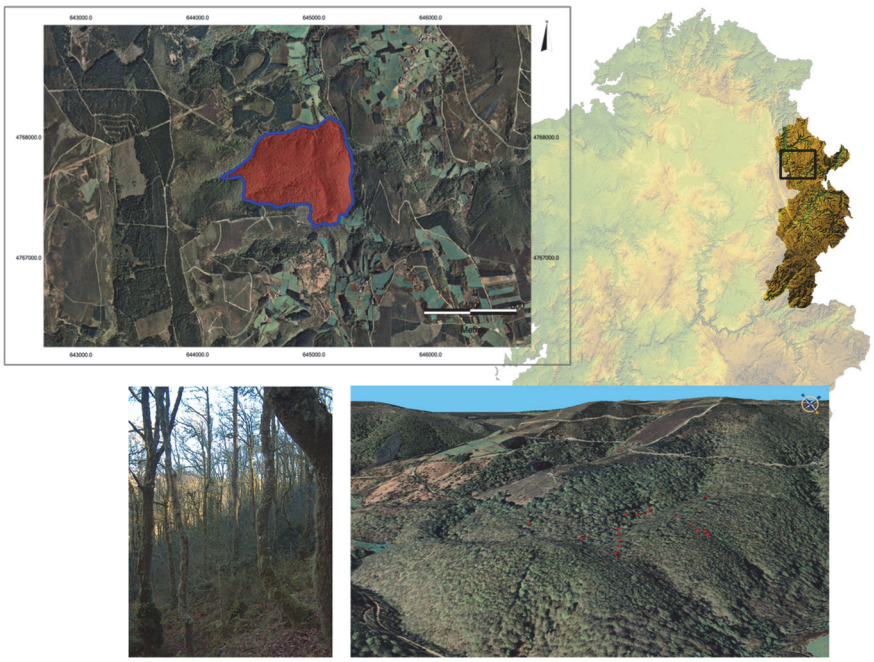


Figura 17. Masa de Estornín. En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados en color rojo.

Estornín (EST)

Este bosque pertenece en su totalidad al ayuntamiento de Baleira, en la comarca de A Fonsagrada. Se localiza en una ladera de umbría entre los 750 y los 850 m de altitud (Figura 17), en una zona de frecuentes nevadas y de pendientes elevadas que han propiciado el buen estado de conservación de la masa. En los diferentes pisos altitudinales aparecen *Quercus robur*, *Quercus pyrenaica*, *Betula alba*, *Taxus baccata*, *Corylus avellana*, *Prunus spinosa* L., *Sorbus aucuparia* e *Ilex aquifolium*. Destaca particularmente la presencia de pies sueltos de *Fagus sylvatica* L., que al igual que en la vecina Fraga de A Marronda o en los montes de A Teixeira, encuentra aquí su límite de distribución suroccidental de Europa. También aparecen matorrales formados por los géneros *Calluna*, *Erica*, *Genista*, *Cytisus*, *Ulex*, etc. allí donde el bosque ha sido eliminado.

Cabanavella (CAB)

Se estudiaron las dos vaguadas del "Monte da Vara", en las que se presenta una masa extensa de *Quercus robur*/*Quercus petraea*, de árboles en general de mediana edad con individuos viejos de grandes dimensiones salpicados entre ellos (Figura 18). Estos últimos presentan en muchos casos frecuentes oquedades en la base del fuste y diferentes daños por rayos e incendios. En las zonas de umbría se mezclan con *Ilex aquifolium* sobre todo y con algunos pies de *Fraxinus excelsior*, *Acer pseudoplatanus* y *Betula alba*, siendo este último más frecuente a medida que se asciende en altitud. En las áreas más insoladas la masa aparece prácticamente pura y con escaso sotobosque, observando matorrales seriales de *Erica australis* en las zonas menos arboladas y más deterioradas por los incendios. Son laderas de fuerte pendiente y difícil acceso por carecer de caminos, hecho que obligó a muestrear monte a través, sobre todo la ladera de umbría.

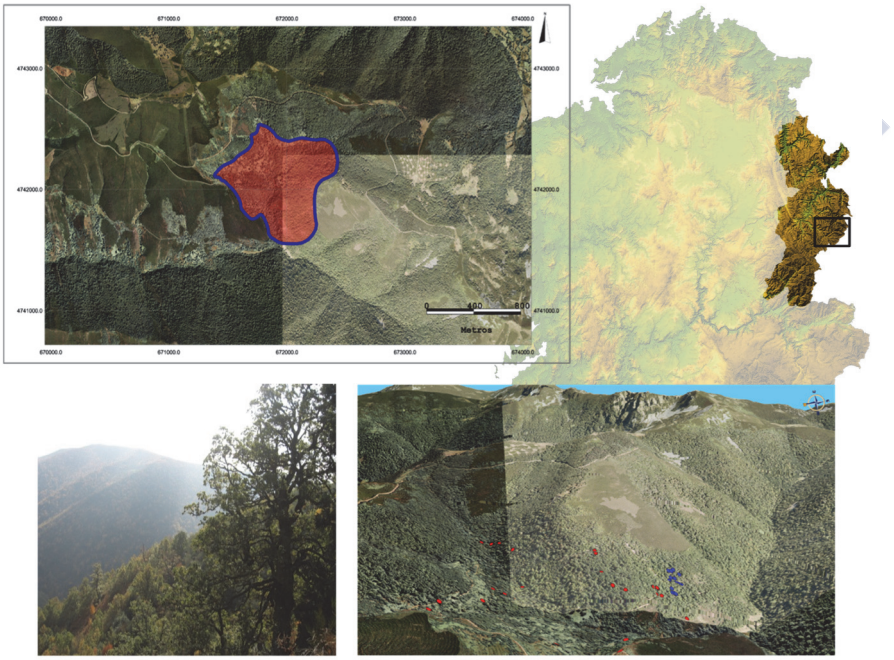


Figura 18. Masa de Cabanavella. En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados, los viejos en color rojo, y los jóvenes en azul.

En esta masa se llevó a cabo un muestreo en el que se recogieron dos tipologías de árbol diferentes. Por un lado, se buscaron los árboles más viejos y de mayores proporciones, lo que obligó a recorrer la totalidad del bosque debido a la elevada dispersión de los pies seleccionados (Figura 18, en rojo); por otro, se buscaron individuos relativamente jóvenes (80-100 años) los cuales proceden de regeneración natural (brinzales) y crecen a lo largo de una ladera de fuerte pendiente y difícil acceso, en

la que apenas hay presencia de sotobosque (Figura 18, en azul). Este monte sufrió una fuerte explotación maderera que finalizó entre 1950 y 1960. Además de esto, las laderas de solana muestreadas sufrieron un incendio hace unos 25 años, del que todavía quedan huellas en los árboles que lo sobrevivieron, y que posiblemente fue la causa de las oquedades mencionadas.

3.1.1.3 Sierras Sudorientales Gallegas

3.1.1.3.1 Localización geográfica

El conjunto comarcal denominado sierras sudorientales se localiza en el centro y nordeste de la provincia de Ourense y el sureste de la provincia de Lugo y constituye una zona de fuertes contrastes físicos y humanos. Ocupa unos 2.581 km^2 y son tierras con una altitud media elevada (>1.000 m) en las que se encuentran los relieves más elevados de Galicia (2.000 m en Pena Trevinca), con la contraposición de la existencia de bloques hundidos, responsables de depresiones como la del río Sil, con tan solo 350 m de altitud. En este entorno se pueden distinguir cuatro comarcas: comarca de Valdeorras, Valle de Quiroga, Terra de Caldelas y comarca de Viana. En las sierras sudorientales se estudiaron tres localidades: dos de ellas, denominadas **A Gudiña** y **Fradelo**, pertenecientes a la comarca de Viana y la otra, denominada **Castro Caldelas**, a Terra de Caldelas.

3.1.1.3.2 Materiales litológicos y suelos

En la zona Galicia Oriental aparecen materiales del paleozoico (pizarras y calcáreas), mientras que en la zona Galicia central-Tras-os-Montes predominan las rocas ígneas (granitos y esquistos) y materiales del precámbrico (formación del Ollo de Sapo). Existen afloramientos de pizarra negra de la serie Luarca del ordovícino medio, depósitos del terciario constituidos por arcilla, arena, conglomerados y grava, restringidos a las márgenes de los ríos, y aluviones y coluviones del cuaternario en las capas superficiales de las depresiones de Valdeorras, el valle de Quiroga y algunos puntos del valle del Bibei. La acción glaciaria y periglaciaria tuvo mucha importancia en las tierras altas, con presencia de depósitos periglaciares en el valle del Sil, en Trevinca y en el macizo de Manzaneda (Álvarez Rodríguez 1996).

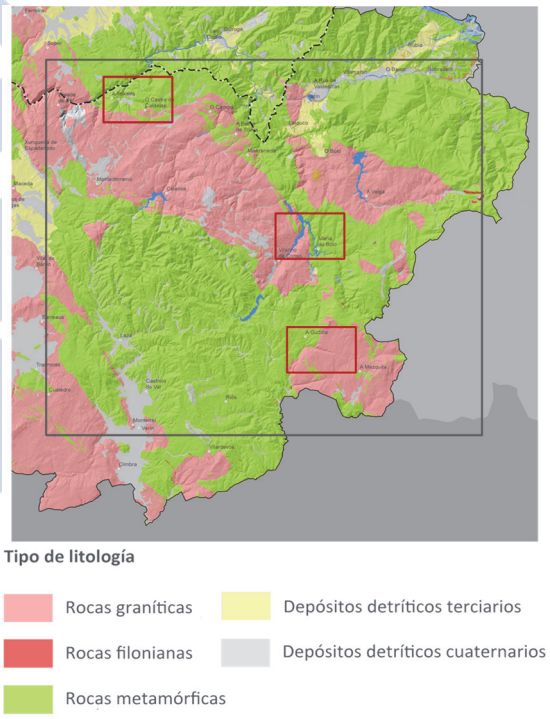


Figura 19. Mapa litológico de las Sierras Sudorientales, con la localización de Castro Caldelas (superior izqda.), Fradelo (centro) y A Gudiña (inferior dcha.) (Fuente: Elaboración propia a partir de cartografía del IGME).

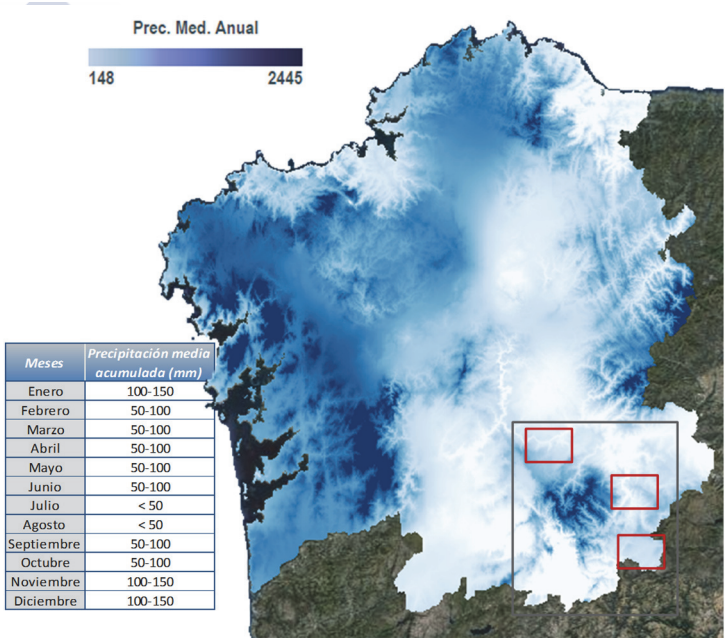
Los suelos presentes en las tres localidades muestreadas son los dominantes en todo el territorio y especialmente en el macizo de Manzaneda, los Leptosoles úmbricos y líticos, y los Regosoles, desarrollados sobre granito, pizarra y esquisto. Los primeros son suelos poco profundos ligados a fuertes pendientes, muy ácidos y pobres, condicionados por la erosión, con escasa capacidad de retención de agua ya que son muy permeables, lo que los hace muy susceptibles a la sequía. Los Regosoles tienen un grado mayor de estabilidad y aparecen todavía en zonas de pendiente pero fundamentalmente en orientaciones norte, más húmedas y sombrías. Es un tipo de suelo más evolucionado y su perfil se caracteriza

normalmente por un horizonte A úmbrico rico en materia orgánica, con un bajo porcentaje de saturación por bases que descansa sobre la roca más o menos alterada (Figura 19).

3.1.1.3.3 Caracterización climática

En estas montañas predomina un clima de tipo suboceánico, caracterizado por la disminución, más o menos acusada según se trate de las sierras o las depresiones, de la influencia oceánica. Debido al relieve accidentado, así como al relativo alejamiento del océano Atlántico, se observan distintas variaciones climáticas. Las sierras actúan de barrera a los frentes oceánicos, que llegan muy atenuados, lo que permite una cierta influencia mediterránea en las depresiones, con temperaturas más elevadas y menor pluviosidad. Estas características, modificadas por la altitud, le reconfieren cierta continentalidad a las tierras altas, con inviernos fríos y húmedos, presencia de nieve persistente durante varios días, y veranos secos y relativamente cálidos. Son muy frecuentes los fenómenos de inversión térmica en los valles durante el invierno, mientras que en verano, debido al calentamiento del suelo por la fuerte insolación, se originan grandes tormentas con fuerte aparato eléctrico o también calimas, nieblas de evaporación producidas por el elevado represamiento de los ríos (Pérez Alberti 1991).

Las precipitaciones medias anuales rondan los 1.200 mm, aunque existen valores extremos en función de la distribución del relieve (Figura 20). Así, en la parte este de Valdeorras, a 355 m de altitud, este valor es de tan solo 594 mm, mientras que este valor en el sur del macizo de Manzaneda, a 1.000 m de altitud, es de 2.240 mm. Exceptuando el macizo de Manzaneda, el resto del territorio refleja las tendencias del clima mediterráneo, con una reducción tanto de los totales pluviométricos como del número de días de lluvia al año, inferior a 120 en algunos puntos (Álvarez Rodríguez 1996). Existe sequía estival al menos durante dos meses, hecho que sitúa en esta zona el déficit hídrico más acusado de Galicia. El gradiente pluviométrico calculado a partir del Atlas Climático (Martínez Cortizas et al. 1999) es de 39 mm/100 m para la localidad de A Gudiña y de 78 mm/100 m para las de Fradelo y Castro Caldelas, con un reparto estacional irregular, representando las precipitaciones estivales el 9 % del total anual. Julio y agosto son los meses más secos, con menos de 50 mm.



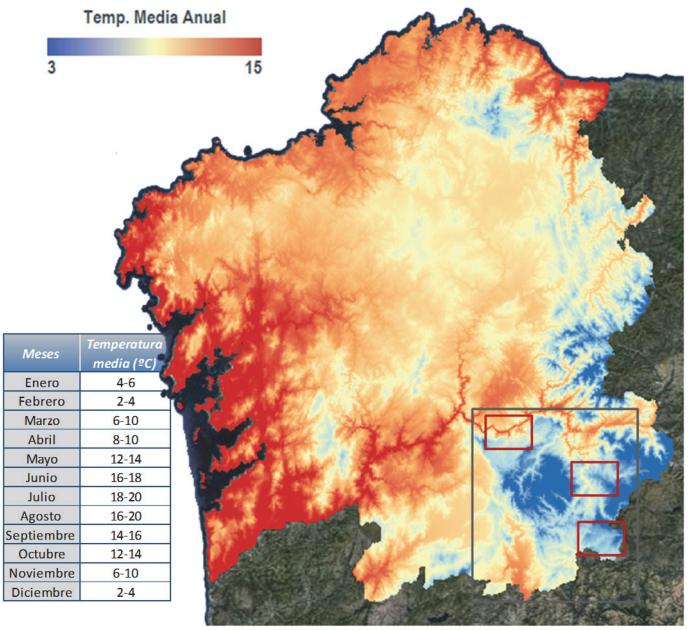
Meses	Precipitación media acumulada (mm)
Enero	100-150
Febrero	50-100
Marzo	50-100
Abril	50-100
Mayo	50-100
Junio	50-100
Julio	< 50
Agosto	< 50
Septiembre	50-100
Octubre	50-100
Noviembre	100-150
Diciembre	100-150

Figura 20. Precipitación media anual (mm) y tabla de rangos de precipitación media mensual acumulada para Castro Caldelas (superior izqda.), Fradelo (centro) y A Gudiña (inferior dcha.)
(Fuente: http://rgis.cesga.es/solos/solos.html).

Las temperaturas están condicionadas por la disposición del relieve, la fragmentación, la lejanía del océano y la altitud, pudiendo establecerse en esta área cuatro zonas con regímenes térmicos perfectamente individualizados (Álvarez Rodríguez 1996). En la depresión del río Sil, por debajo de los 400 m de altitud, se registran temperaturas medias de alrededor de 14 ºC, con una elevada amplitud térmica (16,6 ºC). Los veranos son cálidos y muy largos, y los inviernos son frescos, bajando excepcionalmente de los 0 ºC. Entre los 400-700 m, en los márgenes del valle del Sil y los tramos medio y final de los cursos fluviales del Bibei y sus afluentes, la temperatura media desciende a los 11 ºC debido a la altitud y morfología del relieve. Los veranos no son muy cálidos (solamente julio y agosto se

acercan a los 19 ºC) y los inviernos son fríos y largos, con seis meses por debajo de los 10 ºC y varios días de helada. Entre los 700-1.000 m el matiz mediterráneo es sustituido por el continental, con temperaturas medias anuales de 9 ºC. Los inviernos son muy fríos y los veranos cortos y frescos (solamente julio y agosto se acercan a los 16 ºC), con noches muy frescas en las que se puede llegar a bajar de los 10 ºC. Por encima de los 1.000 m la continentalidad se acentúa, con temperaturas medias que rondan los 7 ºC. Los veranos son frescos (solamente julio supera los 16 ºC), y los inviernos son muy fríos y largos (de noviembre a mayo) (Figura 21).

Para todas las localidades, el gradiente termométrico calculado a partir del Atlas Climático (Martínez Cortizas et al. 1999) es de -0,53 ºC/100 m.



Meses	Temperatura media (ºC)
Enero	4-6
Febrero	2-4
Marzo	6-10
Abril	8-10
Mayo	12-14
Junio	16-18
Julio	18-20
Agosto	16-20
Septiembre	14-16
Octubre	12-14
Noviembre	6-10
Diciembre	2-4

Figura 21. Temperatura media anual (ºC) y tabla de rangos de temperatura media mensual para para Castro Caldelas (superior izqda.), Fradelo (centro) y A Gudiña (inferior dcha.) (Fuente: http://rgis.cesga.es/solos/solos.html).

3.1.1.3.4 Vegetación

En las montañas sudorientales, al igual que en las orientales, tiene lugar la transición entre las regiones Mediterránea y Eurosiberiana. Las tres localidades estudiadas se encuentran incluidas en la región Eurosiberiana; las dos estudiadas en la comarca de Viana (**A Gudiña** y **Fradelo**) en la Provincia Carpetano-Ibérico-Leonesa, Sector Orensano-Sanabriense, Subsector Orensano-Sanabriense (Rivas-Martínez 1987), la tercera localidad, de Terra de Caldelas (**Castro Caldelas)**, en la Provincia Cántabro-Atlántica, Subprovincia Astur-Galaica, Sector Galaico-Portugués, Subsector Xuresiano-Queixense (Rivas-Martínez 1987).

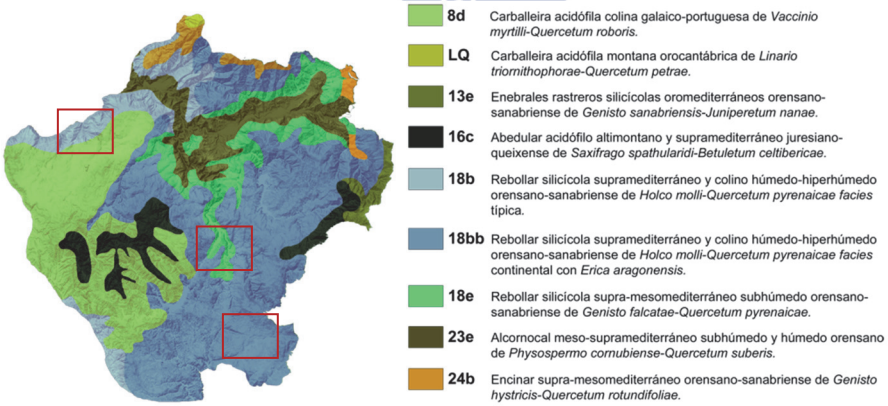


Figura 22. Vegetación potencial de las montañas sudorientales gallegas, con la localización de Castro Caldelas (superior izqda.), Fradelo (centro) y A Gudiña (inferior dcha.) (Fuente: Elaboración propia basado en Rivas-Martínez (1987)).

En las zonas bajas de estas montañas se dan condiciones mediterráneas, apareciendo especies típicas de este clima, como encinas (*Quercus ilex* subsp. *ballota*), alcornoques (*Quercus suber*), olivos (*Olea europea* L.), etc., mientras que en las zonas de alta montaña el clima presenta matices continentales, con presencia de comunidades como los enebrales rastreros de *Genisto sanabriensis-Juniperetum nanae* o los abedulares acidófilos supramediterráneos de *Saxifrago spatularidi-Betuletum celtibericae*. El estrato arbóreo dominante está constituido por diferentes especies de quercíneas, en especial por *Quercus pyrenaica*, más adaptado al clima frío de las zonas altas, aunque estos bosques generalmente se encuentran en regresión tanto por los incendios forestales, que dan paso a comunidades de matorrales seriales como los carpazales o jarales (*Cistus psilosepalus* Aiton), como por las repoblaciones con coníferas.

Los rebollares son las formaciones vegetales estudiadas; en el caso de las localidades de Castro Caldelas y A Gudiña, se corresponde con un **rebollar silicícola supramediterráneo y colino húmedo e hiperhúmedo orensano-sanabriense de *Holco molli-Quercetum pyrenaicae*** (Rivas-Martínez 1987), correspondiendo a la facies continental con *Erica australis* en el caso de A Gudiña. En la localidad de Fradelo, sin embargo, existe un **rebollar silicícola supra-mesomediterráneo subhúmedo orensano-sanabriense de *Genisto falcatae-Quercetum pyrenaicae*** (Rivas-Martínez 1987) (Figura 22). En ambas asociaciones domina *Quercus pyrenaica*, que forma un estrato arbóreo de elevada cobertura y altura de 10-15 m, con árboles de pequeño diámetro, muchos de ellos provenientes de rebrotes de cepa. El estrato arbustivo tiene cobertura baja y altura de 1-3 m, en que se encuantran rebollos jóvenes mezclados con diversas especies arbustivas. El estrato herbáceo tiene también alta cobertura, con numerosas especies rizomatosas y cespitosas de carácter acidófilo. Los suelos sobre los que se asientan corresponden a Leptosoles úmbricos, Cambisoles húmicos o dístricos, con humus evolucionado y pH superior a 5, en los que a veces aparecen fenómenos de podsolidación. Se sitúan sobre pizarras, granitos, cuarcitas ó calizas, en laderas de pendiente variable y altitudes de hasta 1.300 m. En los montes de Trevinca aparece el tejo (*Taxus baccata*) como especie acompañante del rebollo, mientras que el haya (*Fagus sylvatica*) es relativamente abundante en varias localidades de la zona de O Bolo, en la sierra de Queixa o en los montes do Invernadeiro.

En la asociación *Holco molli-Quercetum pyrenaicae* se incorporan especies submediterráneas como *Cirsium palustre* (L.) Scop., *Asplenium adiantum-nigrum* L., *Cytisus multiflorus* (L´Hér.) Sweet, *Narcissus pseudonarcissus* L., *Rubia peregrina* L. u *Origanum vulgare* L., con la presencia además de *Erica australis* en la variante presente en la zona de A Gudiña. En la composición florística de la asociación *Genisto falcatae-Quercetum pyrenaicae* desaparecen los elementos atlánticos y son sustituidos por especies como *Genista falcata* Brot., *Festuca elegans* Boiss ó *Luzula forsteri* (Sm.) Dc. (Silva Pando & Rigueiro Rodríguez 1992).

3.1.1.3.5 Localidades muestreadas

En la comarca de Viana se estudiaron dos masas de *Quercus pyrenaica* denominadas **A Gudiña (GUD)** y **Fradelo (FRA)** por encontrarse cercanas a las poblaciones de A Gudiña y en pleno embalse del Bao, municipio de Fradelo, respectivamente. La tercera masa de *Q. pyrenaica*, seleccionada en la comarca de Terra de Caldelas, se identificó como **Castro Caldelas (CAL)** por encontrarse en una ladera muy cercana a la villa del mismo nombre.

A Gudiña (GUD)

Se trata de un bosque situado en una ladera de elavada pendiente, encajado en un valle alargado y que ocupa una superficie de alrededor de 80 ha, muy cercano a la población de A Gudiña (Figura 23). Se encuentra a una altitud de entre 850-1.050 m. y la especie predominante es *Quercus pyrenaica*, que crece sobre un suelo desarrollado a partir de granito moscovítico de grano fino, aunque en el valle aparecen con relativa frecuencia *Fraxinus angustifolia* y *Corylus avellana* siempre ligados al arroyo que discurre por el fondo del valle.

Es una masa en la que existe bastante humedad, encontrándose la zona de muestreo en la ladera orientada al Norte. La ladera opuesta se encuentra poblada por el mismo tipo de formación, que desaparece a partir de la mitad de la misma para dar paso a matorrales seriales de *Erica* y *Cytisus* con claras evidencias de incendios recurrentes. En la zona de muestreo hay evidencias de haber sufrido también incendios relativamente recientes, ya que aparecen numerosos pies con cicatrices longitudinales, así como tocones y árboles superficialmente carbonizados. El sotobosque de esta localidad se compone de especies como *Lonicera peryclimenum, Primula vulgaris* Huds.*, Pteridium aquilinum, Rubus sp., Omphalodes nitida* Hoffmanns. & Link*, Melittis melissophyllum* L.*, Melampyrum pratense* L.*, Galium odoratum* (L.) Scop.*, Erica arborea, Cytisus scoparius, Cytisus multiflorus, Dryopteris affinis, Blechnum spicant, Crataegus monogyna* Jacq.*, Pterospartum tridentatum, etc.*

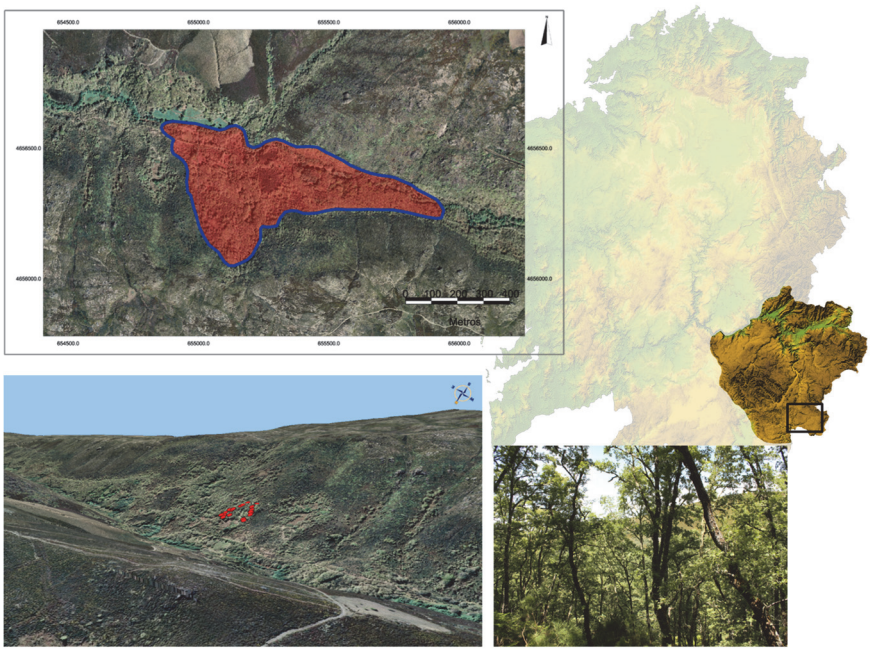


Figura 23. Masa de A Gudiña. En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados en color rojo.

Fradelo (FRA)

Se trata de una masa situada en la ladera de un antiguo viñedo y un "souto" (plantación de castaños para aprovechamiento del fruto), donde todavía son visibles los restos de los muros del aterrazado que existía en toda la ladera. Este hecho denota un intenso aprovechamiento en el pasado, si bien en la actualidad este rebollar no acusa signos de ningún tipo de intervención humana, salvo la extracción ocasional de leñas muertas.

Se encuentra emplazada a orillas de uno de los brazos del embalse del Bao (Figura 24), muy cerca de Viana do Bolo y en los terrenos de la aldea conocida como Fradelo, a una altitud de entre 650-800 m, en una zona de elevada pendiente. Su estructura es de un estrato compuesto por árboles de sebe (árboles que se utilizaban para delimitar los distintos predios) de buenas proporciones, a partir de los cuales se ha regenerado la masa en toda la ladera estudiada, tratándose de un regenerado denso que ha dado lugar a un bosque de cobertura media con un sotobosque compuesto por *Teucrium scorodonia* L.*, Crataegus monogyna, Galium aparine* L.*, Genista falcata* Brot.*, Cistus salviifolius* L.*, Cistus multiflorus, Pteridium aquilinum, Rubus sp., Lonicera peryclimenum, Stellaria holostea* L.*, Cytisus scoparius, Rumex sp., Erica arborea, Prunus avium* L.*, Viola riviniana* en la parte más húmeda de la masa*, Brachypodium sylvaticum* (L.) (Hudson) Beauv.*, Holcus mollis* L., etc. En algunos puntos aparecen viejos pies de *Castanea sativa*, así como algún individuo juvenil de *Quercus robur*.

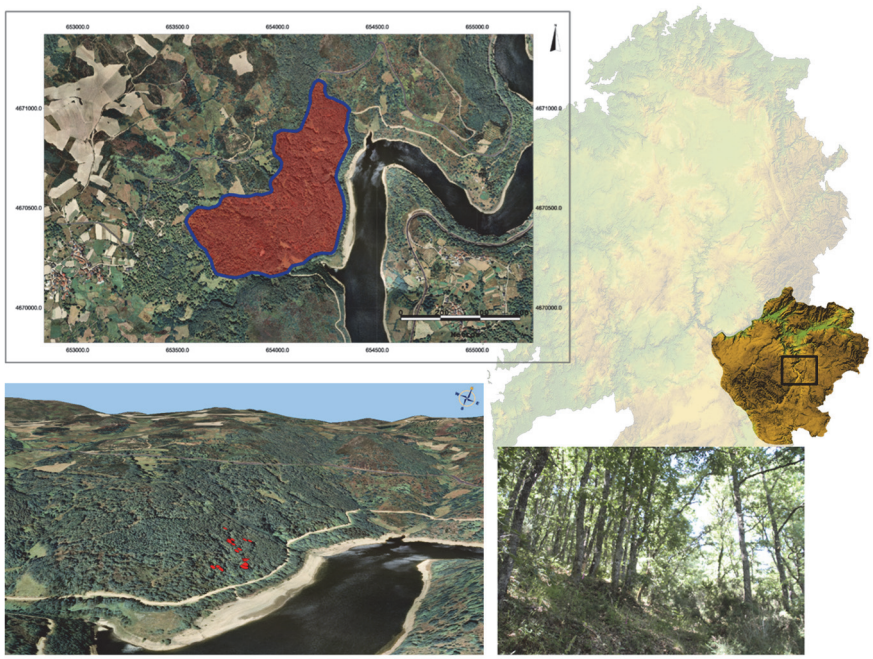


Figura 24. Masa de Fradelo. En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados en color rojo.

Castro Caldelas (CAL)

Se sitúa en unas laderas de fuerte pendiente, por cuyo valle discurre el río Edo, en las inmediaciones de la villa de Castro Caldelas (Figura 25). En estas laderas la vegetación cambia drásticamente según la orientación de las mismas, la presencia o ausencia de cursos de agua estacionales y el grado de proximidad al río. Se trata de una masa de gran extensión que se encuentra entre 430-725 m. de altitud, dominada principalmente por *Quercus pyrenaica*, con presencia de algunos pies de *Quercus robur* tanto de grandes proporciones como juveniles en las zonas menos insoladas.

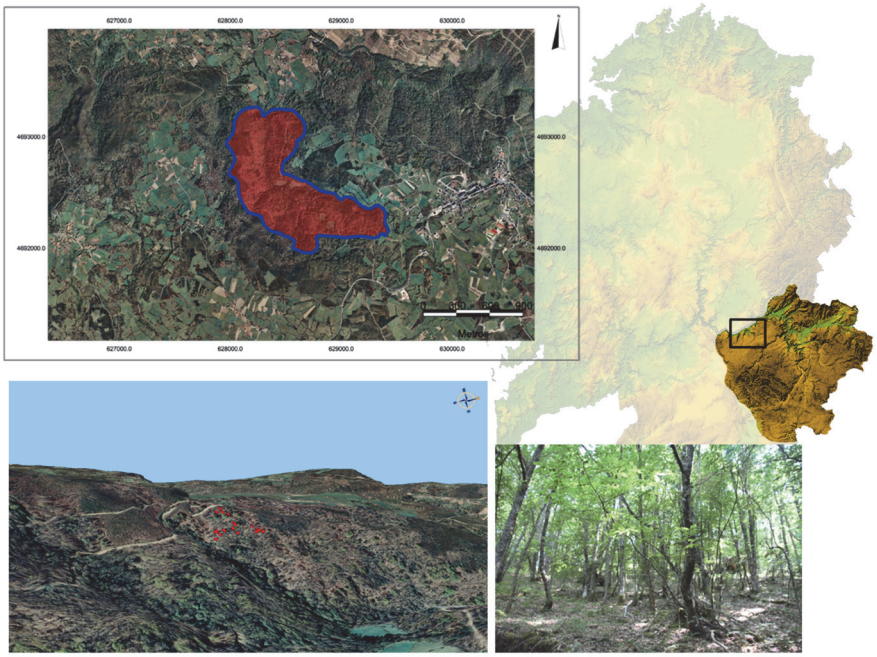


Figura 25. Masa de Castro Caldelas. En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados en color rojo.

En las partes más húmedas de la masa aparecen numerosos individuos de *Castanea sativa* maduros de grandes proporciones, formando parte de soutos en estado de abandono, vestigios de las antiguas dehesas que existían en el pasado en este territorio. En estas zonas húmedas aparecen, como vegetación acompañante *Acer pseudoplatanus, Betula alba, Asplenium adiantum-nigrum* L.*, Dryopteris affinis*, *Polystichum aculeatum* (L.) Roth, etc. En las partes más secas aparece una masa pura de *Quercus pyrenaica* con pies de proporciones modestas que dotan de una cobertura media, en la que el sotobosque está compuesto por *Cytisus multiflorus, Melampyrum pratense, Ruscus aculeatus* L.*, Pteridium aquilinum, Asphodelus albus* Mill.*, Teucrium scorodonia, Anthoxanthum odoratum* L.*, Hedera helix* L.*, Pseudarrhenatherum longifolium* (Thore) Rouy*, Lonicera periclymenum, Genista falcata, Ulex minor* Roth.*, Rubus sp., Cytisus scoparius, Cytisus striatus, Marrubium vulgare* L.*, Lithodora postrata* (Loisel) Griseb.*, Primula vulgaris, Hypericum pulchrum* L.*,* etc.

3.1.1.4 Depresiones terciarias interiores de Ourense y Lemos.

3.1.1.4.1 Localización geográfica

Un elemento fundamental en la configuración del relieve gallego es la presencia de una serie de fosas tectónicas localizadas tanto en la costa como en el interior. Se trata de segmentos del territorio hundido que aparecen flanqueados por sierras, los cuales forman un amplio abanico de valles fluviales que, por regla general, transcurran encajados entre las sierras. Esta situación de alternancia entre diversas formas de relieve explica la gran variabilidad climática de la región (Martínez Cortizas et al. 1999). Dentro de las depresiones interiores, son las de Ourense y Lemos las que se han seleccionado como zonas de muestreo. La depresión de Ourense engloba varios municipios que se organizan administrativamente en las comarcas de Ourense, Allariz-Maceda, O Carballiño, O Ribeiro y Terra de Celanova. La depresión de Lemos incluye municipios que pertenecen a las comarcas lucenses de Sarria y Terra de Lemos fundamentalmente.

Las localidades estudiadas en la Depresión de Ourense, **Ramirás** y **Vilanova dos Infantes**, se encuentran en el extremo occidental de la provincia, en la Comarca de *Terra de Celanova*, la cual se inscribe en una depresión topográfica marcada de este a oeste por el curso medio del río Arnoia, y de sur a norte por el que trazan, en paralelo, sus principales afluentes, el Tuño y el Sorga. Ocupa una superficie de unos 508 km² y presenta un relieve suave, de formas aplanadas y elevaciones que conformaron este gran valle.

Las ubicaciones estudiadas en la Depresión de Lemos, **Sober** y **Villaoscura**, están en el extremo sur de la provincia de Lugo, en la Comarca de *Terra de Lemos*, que en la parte occidental se encuentra con el río Miño como barrera natural con la comarca de Chantada. Ocupa alrededor de 940 km² y cuenta con varias formas de relieve, con las mayores altitudes hacia el sur (750 m) en el valle del río Sil, que da paso a la depresión de Monforte, mientras que al norte aparece el valle formado por el río Cabe.

3.1.1.4.2 Materiales litológicos y suelos

Una parte importante de los materiales que aparecen distribuidos por este sector corresponden al denominado dominio esquistoso de Galicia-Tras-os-Montes, con una extensión coincidente con la dirección que muestran las estructuras hercínicas. Se compone fundamentalmente de micaesquistos, esquistos cuarzosos y feldespáticos, y paragneises con intercalaciones de rocas ortoderivadas ácidas de origen volcánico o subvolcánico, aunque también se observan intercalaciones de rocas grafitosas, cuarcitas y anfibolitas. Estos conjuntos corresponden a distintas áreas paleogeográficas de una cuenca de sedimentación desarrollada sobre una superficie continental antigua, y están tectónicamente superpuestos. En la base de los materiales que configuran este dominio se sitúan ortogneises

glandulares, que afloran en un área de intensa migmatización y que muestran inyecciones graníticas abundantes (Fumega Piñeiro 1996).

Los suelos desarrollados en las zonas de estudio proceden de granitos y esquistos (Figura 26), son ácidos y presentan generalmente buen contenido de materia orgánica. En ambas localizaciones aparecen tierras pardas mesotróficas en los fondos de valle y superficies de aplanamiento, con un color rojizo o pardo-oscuro y un perfil con una transición gradual entre los diferentes horizontes. Son suelos sueltos, sin estructura y con buen drenaje, con problemas puntuales de encharcamiento derivados de la topografía como consecuencia de su localización en áreas de escasa pendiente. También hay presencia de Cambisoles asociados a granitos, con escaso contenido en arcilla y una tendencia a la granulometría arenosa que facilita el drenaje. En las zonas de pendientes elevadas y donde los incendios han destruido recurrentemente la cubierta vegetal aparecen afloramientos rocosos, y ligados a ellos, suelos muy someros con un horizonte orgánico poco desarrollado y sustentado por una vegetación de escasa calidad (Fumega Piñeiro 1996). Bajo estas condiciones aparecen Leptosoles úmbricos, que suponen el estadio evolutivo final de los suelos poco evolucionados hacia las tierras pardas. Tienen un horizonte orgánico bien humificado seguido, a veces, por un horizonte B de alteración poco desarrollado, generalmente presentan una porción importante de materia orgánica, son permeables y están bien drenados.

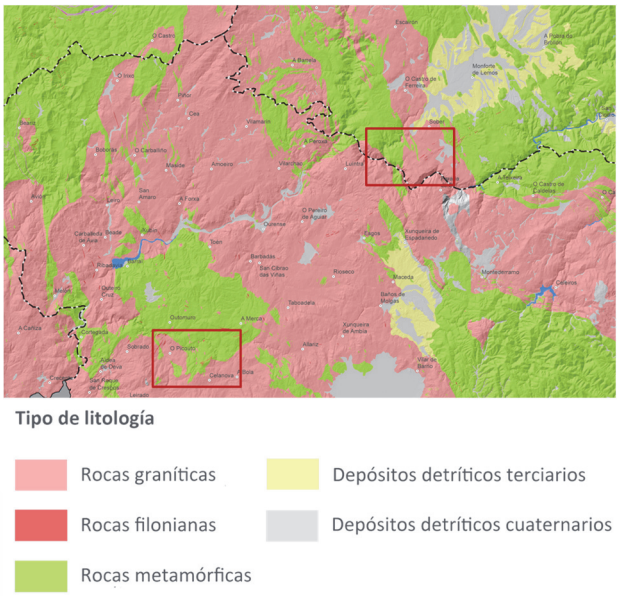
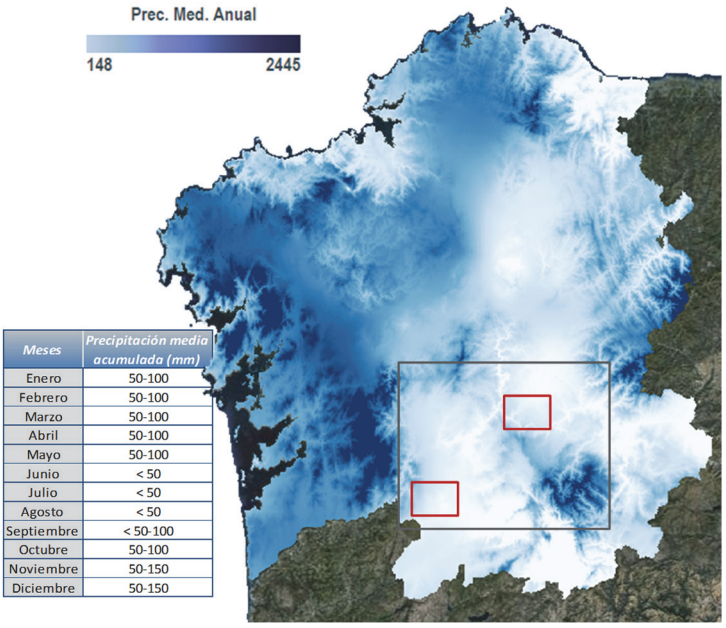


Figura 26. Mapa litológico del entorno de las depresiones terciarias de Ourense y Lemos, con las zonas estudiadas en la Comarca de Celanova (inferior izqda.) y en la Comarca de Lemos (superior dcha.) (Fuente: Elaboración propia a partir de cartografía del IGME).

3.1.1.4.3 Caracterización climática

Dada la distancia del litoral y sobre todo debido a que la Dorsal Gallega (Sierra del Suido, Sierra del Faro de Avión y Montes del Testeiro) ejerce de pantalla pluviométrica de los vientos cargados de humedad, el clima en estas dos amplias áreas del interior gallego tiene un carácter continental con tendencia mediterránea (Martínez Cortizas et al. 1999). En estas zonas se produce una disminución más brusca de las temperaturas mínimas estacionales del verano al otoño que en las sierras. Sin embargo, las temperaturas máximas registran una disminución menor en los valles en esta época. Por otro lado, la existencia de sequía estival durante los meses de julio y agosto condiciona



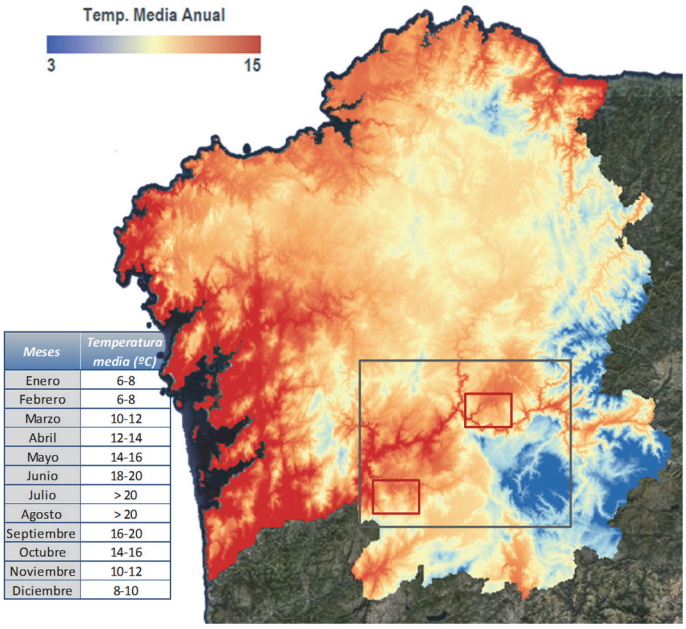
Meses	Precipitación media acumulada (mm)
Enero	50-100
Febrero	50-100
Marzo	50-100
Abril	50-100
Mayo	50-100
Junio	< 50
Julio	< 50
Agosto	< 50
Septiembre	< 50-100
Octubre	50-100
Noviembre	50-150
Diciembre	50-150

Figura 27. Precipitación media anual (mm) y tabla de rangos de precipitación media mensual acumulada para la Comarca de Celanova (izqda.) y de Lemos (dcha.).
(Fuente: http://rgis.cesga.es/solos/solos.html).

notablemente la disponibilidad hídrica en dichas áreas (Fumega Piñeiro 1996).

Las precipitaciones son moderadas a lo largo del año con la excepción de los meses de julio, agosto y, en algún caso, incluso el mes de septiembre, en los que se producen frecuentes situaciones de sequía estival (Figura 27). Esta situación determina una fuerte estacionalidad. La precipitación media anual en ambas depresiones es moderada o escasa, alcanzando valores medios máximos inferiores a los 600 mm. El gradiente pluviométrico calculado según el Atlas Climático (Martínez Cortizas et al. 1999) es de 75 mm/100 m, apareciendo un mínimo estival con algún mes seco, con precipitaciones inferiores a los 50 mm.

En ambas depresiones, **las temperaturas** presentan características muy similares (Figura 28), con veranos templados e inviernos suaves, pero con presencia de heladas tardías frecuentemente. Las temperaturas medias más altas se registran en julio y agosto, superando ampliamente los 20 ºC en ambas localizaciones. Presenta un gradiente térmico según los datos del Atlas Climático (Martínez Cortizas et al. 1999) de -0,53 ºC/100 m.



Meses	Temperatura media (ºC)
Enero	6-8
Febrero	6-8
Marzo	10-12
Abril	12-14
Mayo	14-16
Junio	18-20
Julio	> 20
Agosto	> 20
Septiembre	16-20
Octubre	14-16
Noviembre	10-12
Diciembre	8-10

Figura 28. Temperatura media anual (ºC) y tabla de rangos de temperatura media mensual para la Comarca de Celanova (izqda.) y de Lemos (dcha.).
(Fuente: http://rgis.cesga.es/solos/solos.html).

3.1.1.4.4 Vegetación

Ambas depresiones forman parte de la zona de transición entre las regiones biogeográficas Mediterránea y Eurosiberiana. Las dos localidades estudiadas en la depresión de Ourense,en la comarca de Terra de Celanova, pertenecen claramente a la Región Eurosiberiana, Provincia Cántabro-Atlántica, Subprovincia Astur-Galaica, Sector Galaico-Portugués, Subsector Miñense (Rivas-Martínez 1987). Por el contrario, las localidades estudiadas en la depresión de Lemos, en la comarca de Lemos, se incluyen en la Región Mediterránea, Provincia Carpetano-Ibérico-Leonesa, Sector Orensano-Sanabriense, Subsector Orensano-Sanabriense (Rivas-Martínez 1987).

En los dominios mediterraneizados de los valles del Miño y sus afluentes, los bosques climácicos predominantes están constituidos por *Quercus pyrenaica*, que aparece acompañado por especies como *Erica cinerea*, *Anemone trifolia* L., *Castanea sativa, Physospermum* sp., etc. Estos bosques aparecen mezclados con asociaciones dominadas por *Quercus robur* en las zonas de ecotonía, mezclándose los elementos de ambas formaciones. En general, se puede establecer que en las zonas de vaguada y umbrías abundan las formaciones dominadas por *Quercus robur*, mientras que en las laderas soleadas del valle del Miño-Sil y en los alrededores de Ourense, así como en la totalidad de la depresión de Lemos, es *Quercus pyrenaica* la especie dominante (Fumega Piñeiro 1996).

En las dos localidades emplazadas en la depresión de Ourense (Terra de Celanova) se estudió una formación clasificada, según Rivas-Martínez (1987), como una ***carballeira acidófila colina galaico-portuguesa*** **de** ***Rusco aculeati-Quercetum roboris*** (Figura 29). Se trata de una asociación vegetal común en el oeste de Galicia, en localidades con un período de sequía estival no demasiado

pronunciado. Se desarrollan sobre rocas silíceas como granitos que dan lugar a suelos oligotróficos o eutróficos no podsolizados, con humus evolucionado y baja acidez. Su estructura presenta un estrato arbóreo con cobertura del 50% ó mayor, donde la especie que domina es *Quercus robur* acompañado de *Laurus nobilis* L., *Ilex aquifolium*, *Crataegus monogyna*, *Arbutus unedo*.L, *Castanea sativa*, etc. El sotobosque suele estar compuesto por *Pteridium aquilinum*, *Hedera helix*, *Lonicera peryclimenum*, *Ulex minor*, *Ulex galli* Planch., *Ruscus aculeatus*, *Holcus mollis*, *Erica umbellata*, entre otros (Silva Pando & Rigueiro Rodríguez 1992).

Por otra parte, en las dos localidades de la depresión de Lemos (Terra de Lemos), la formación estudiada se corresponde con un **rebollar silicícola supramediterráneo y colino húmedo-hiperhúmedo orensano-sanabrense de *Holco molli-Quercetum pyrenaicae* facies típica** (Rivas-Martínez 1987) (Figura 29). Este tipo de formación aparece en el oriente y sur de la provincia de Ourense y sureste de la de Lugo, en la que algunas especies del ámbito atlántico son sustituidas por especies supramediterráneas. Se sitúan estos rebollares sobre suelos clasificados como Leptosoles úmbricos (grises y pardos), Cambisoles, con humus evolucionado y pH superior a 5, con presencia a veces de fenómenos de podsolidación. Están emplazados sobre pizarras, granitos, cuarcitas o calizas. Se suelen encontrar en laderas de pendiente variable y en cualquier exposición (Silva Pando & Rigueiro Rodríguez 1992).

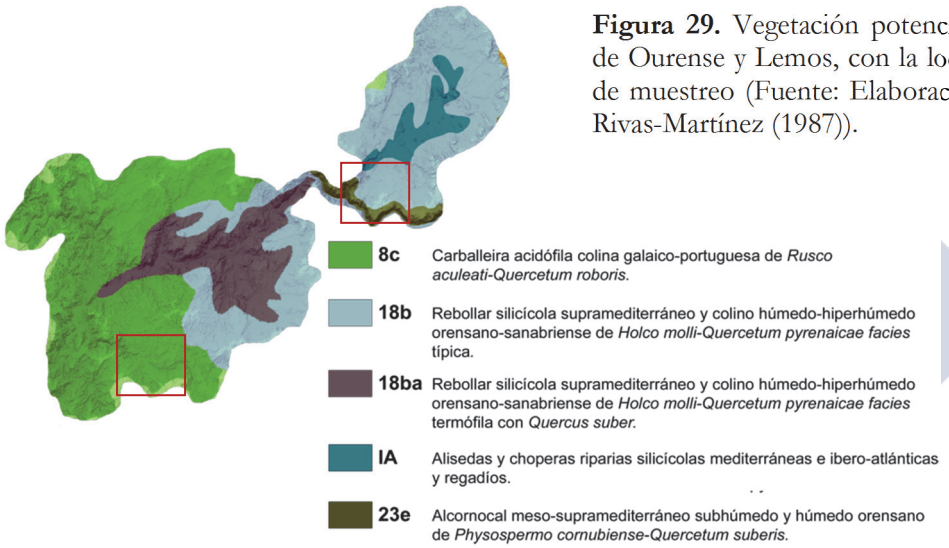


Figura 29. Vegetación potencial de las depresiones de Ourense y Lemos, con la localización de las áreas de muestreo (Fuente: Elaboración propia basado en Rivas-Martínez (1987)).

Como consecuencia de la degradación de esta vegetación potencial, generalmente por causa de los incendios recurrentes, el paisaje vegetal dominante está constituido por brezales y tojales, con presencia de *Calluna vulgaris, Ulex minor, Ulex europaeus, Cytisus striatus, Sarothamnus scoparius*, etc., formaciones con gran interés como protectoras del suelo. Las plantaciones puras o mixtas de *Pinus pinaster* son dominantes por debajo de los 700 m de altitud. Son frecuentes en todo el territorio las acacias (*Acacia dealbata* Link) especie invasora introducida con fines ornamentales y de fijación de taludes que en la actualidad se encuentra muy extendida, sobre todo en los valles vinícolas debido a su uso en el estacado de las viñas.

3.1.1.4.5 Localidades muestreadas

En la Depresión de Ourense (Comarca de Terra de Celanova), se muestrearon dos masas de *Quercus robur*, que se denominaron **Ramirás (RAM)** y **Vilanova dos Infantes (INF)**, situadas en la mitad norte de dicha comarca. La primera de ellas se encuentra en el ayuntamiento de Ramirás, y la segunda se encuentra muy cerca de la villa medieval de Vilanova dos Infantes.

Ramirás (RAM)

Localizada en una vaguada próxima al pueblo de Vilaboa de Paizás, perteneciente al municipio de Ramirás, se trata de una masa monoespecífica de *Quercus robur* con individuos de tamaño intermedio, que está rodeada de prados y repoblaciones forestales de *Pinus pinaster* (Figura 30). La distribución de pies es bastante regular en la parcela, tratándose de una antigua *carballeira* de la que se extraía leña y madera de pies completos y se utilizaba también como zona de pasto para ganado bovino durante la época estival. La vegetación de sotobosque también era aprovechada como cama para el ganado, por lo que se trata de una masa bien conservada con pies de avanzada edad, muy altos y sin evidencias de podas o desmoches.

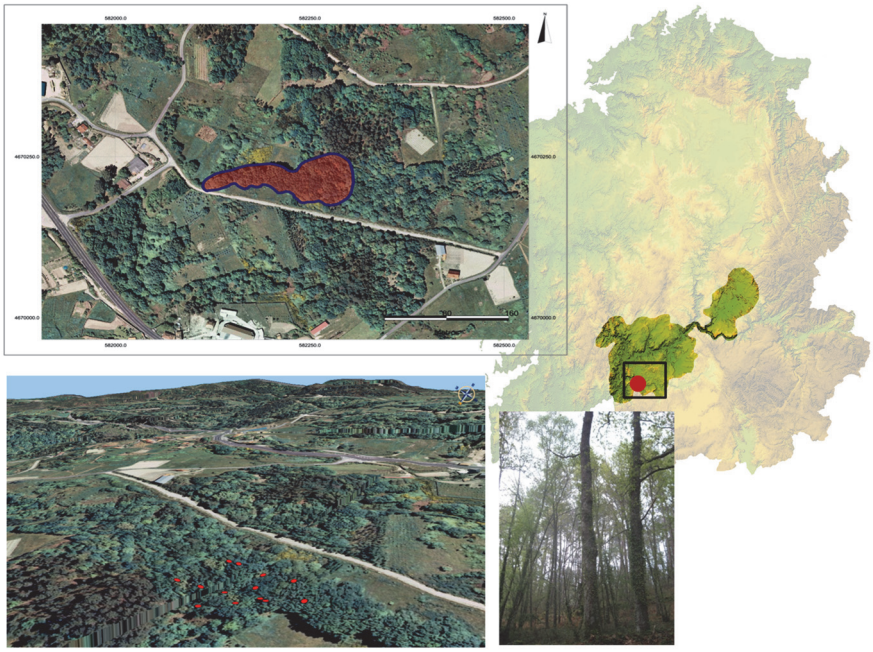


Figura 30. Masa de Ramirás. En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados en color rojo.

Como especies de sotobosque aparecen *Ruscus aculeatus*, *Lonicera periclymenum*, *Teucrium scorodonia*, *Hedera helix*, *Castanea sativa*, *Asplenium onopteris* L., *Tamus communis* L., *Laurus nobilis* L., *Hypericum pulchrum*, *Frangula alnus* Mill., *Physospermum cornubiense* (L.) DC., *Prunus avium*, *Arbutus unedo*, *Rubia peregrina* L., *Pteridium aquilinum*, *Rubus* sp., *Polypodium gr. cambricum* L. subsp. *Cambricum, etc.*.

Vilanova dos Infantes (INF)

Próxima al núcleo medieval de Vilanova dos Infantes, se trata de una masa pura de *Quercus robur,* ubicada en la parte medio-alta de una ladera orientada al sur-sureste, con árboles de considerables dimensiones (Figura 31). Aunque la proporción de árboles de avanzada edad es bastante alta y se encuentran distribuidos de manera regular por la totalidad de la parcela, se puede apreciar que antiguamente eran terrenos de cultivo en los que la regeneración natural partió de pies que conformaban *sebes*, fácilmente reconocibles en el terreno por sus grandes dimensiones y la evidencia de antiguos y recurrentes desmoches, además de estar dispuestos en hileras, paralelos a los restos de antiguos muros de piedra. La composición florística del sotobosque es muy similar a la localidad de Ramirás, dominando *Lonicera periclymenum*, *Rubus sp.*, *Urtica sp.*, *Ruscus aculeatus* y *Hedera helix*. Aparece *Genista florida* L. en

menor proporción, y *Acacia dealbata* en las zonas más degradadas de la parcela o en las zonas de arbolado menos denso.

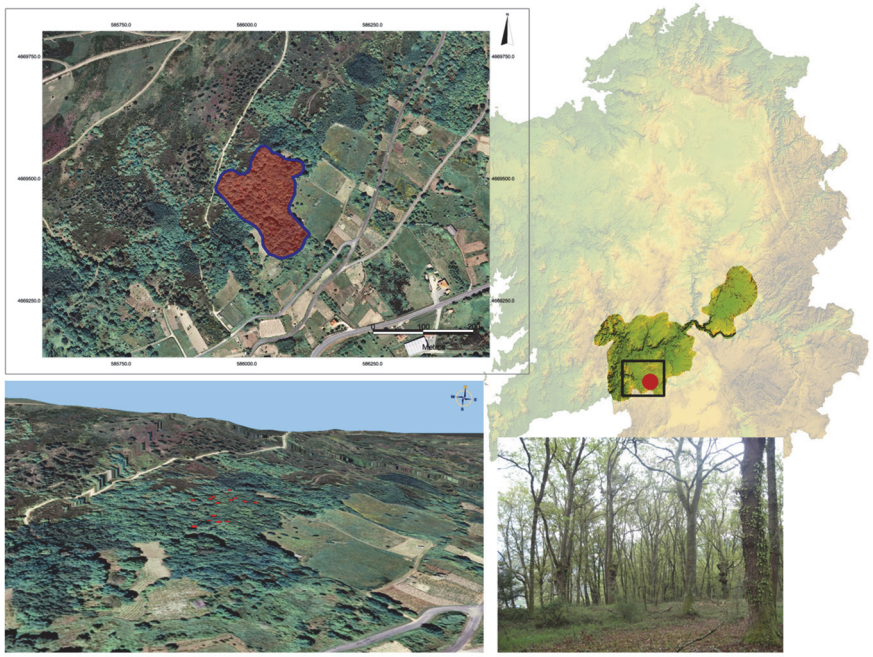


Figura 31. Masa de Vilanova dos Infantes. En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados en color rojo.

En la Depresión de Lemos (Comarca de Terra de Lemos), se muestrearon dos formaciones vegetales pobladas por *Quercus pyrenaica*, que se denominaron **Sober (SOB)** y **Villaoscura (VIO)**. La primera de ellas se encuentra en la zona conocida como A Carqueixa, mientras que la segunda está muy cerca del pueblo de Villaoscura, ambas en el Ayuntamiento de Sober.

Sober (SOB)

Próxima a la zona denominada A Carqueixa, la cual se caracteriza por tener una mínima pendiente, en esta masa los árboles son de mediano tamaño y se distribuyen regularmente conformando una masa monoespecífica de *Quercus pyrenaica*, si bien algunos de los pies más grandes sí que aparecen alineados en los límites de diferentes predios, constituyendo sebes (Figura 32). Se trata de una masa más o menos abierta con presencia de numerosos prados a modo de pequeñas manchas dispersas en el interior, en la que aparece un sotobosque compuesto por *Rubus sp., Poa nemoralis* Boiss., *Athyrium filix-femina* (L.) Roth. Soros., *Polystichum setiferum, Hypericum androsaemum* L., *Lysimachia vulgaris* L., *Holcus mollis, etc.*

Villaoscura (VIO)

Localizada en la zona más alta de una de las laderas que descienden al río Cabe, en la que la pendiente se suaviza mucho en comparación al resto de la ladera.

Es el enclave de un antiguo castro (Castro de Villaoscura) sobre el que se extiende una gran masa de *Quercus pyrenaica* relativamente joven y de una densidad bastante elevada, con presencia de algunos pies sueltos de *Quercus robur* en la zona de borde. Debido a la extensión y escasa edad de la masa y a la abundancia de individuos con oquedades o podredumbres en el fuste, fue necesario recorrer la masa en su totalidad para buscar suficiente número de pies aptos para el muestreo. Esto permitió identificar un rodal no demasiado extenso de pies de buenas dimensiones y presumible avanzada edad

del que, probablemente, partió el regenerado de toda la zona (Figura 33). El sotobosque es muy similar al que aparece en Sober, con especies como *Rubus sp., Cytisus multiflorus*, *Poa nemoralis, Athyrium filix-femina, Polystichum setiferum, Hypericum androsaemum, Holcus mollis, Brachypodium sylvaticum, etc.*

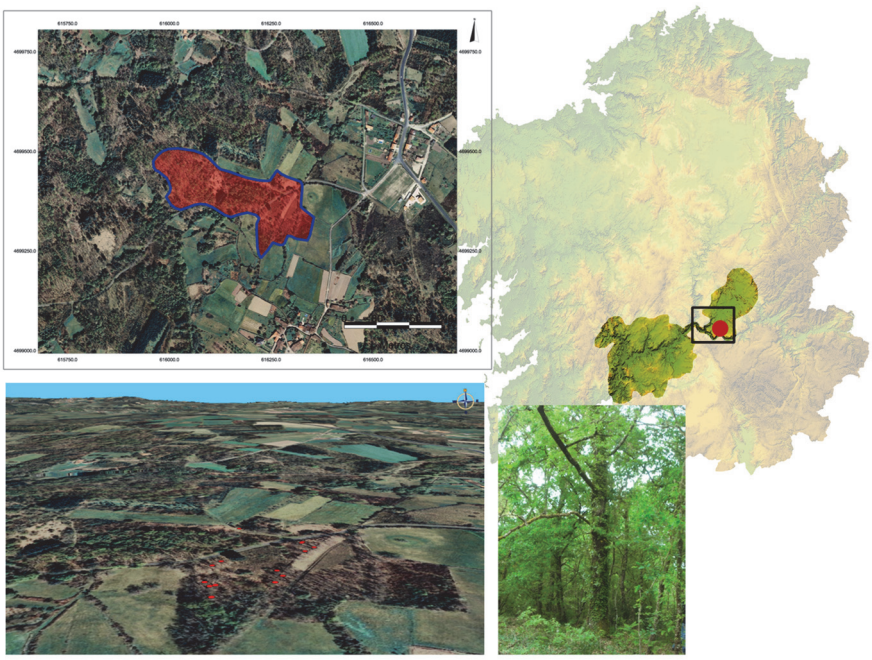


Figura 32. Masa de Sober. En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados en color rojo.

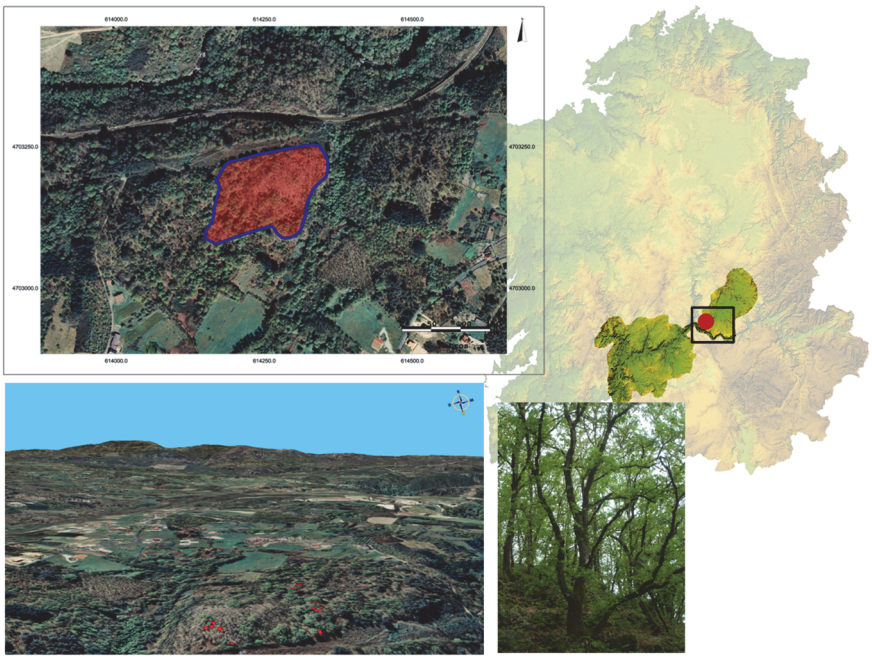


Figura 33. Masa de Villaoscura. En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados en color rojo.

3.1.2 Comarca de Sanabria

La Comarca de Sanabria se sitúa en el noroeste de la provincia de Zamora, en la Comunidad de Castilla y León. Ocupa 1.216 km^2, que constituye el 11,15% del total de la provincia. Limita al norte con la provincia de León, al sur con la provincia portuguesa de Trás-os-Montes e Alto Douro, al este con la comarca zamorana de La Carballeda, y al oeste con la provincia gallega de Ourense. Parte de sus municipios, concretamente Cobreros, Galende, Porto y Trefacio, forman parte del Parque Natural del Lago de Sanabria, así como Pedralba de la Pradería y Puebla de Sanabria forman parte del Parque Natural de la Sierra de la Culebra.

Las dos localidades estudiadas en esta comarca se encuentran dentro del Parque Natural del Lago de Sanabria y Alrededores (Figura 34).

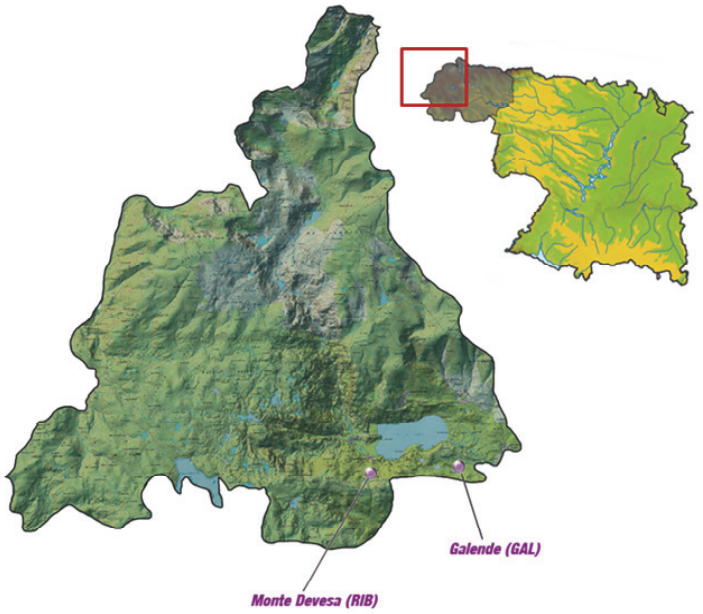


Figura 34. Situación del Parque Natural del Lago de Sanabria y Alrededores, y de las localidades muestreadas.

3.1.2.1 Parque Natural del Lago de Sanabria y Alrededores.

3.1.2.1.1 Localización geográfica

El área de estudio correspondiente al Parque del Lago de Sanabria y Alrededores se sitúa en el extremo noroccidental de la provincia de Zamora (Castilla y León), entre las sierras de Segundera, Cabrera Baja y Culebra. Se encuadra dentro del término municipal de Galende, entre 42º 07' / 42º 05' de latitud Norte y 6º 44' / 6º 38' de longitud Oeste. Se trata de un territorio en el que, debido a la importancia de sus valores naturales y ecológicos, se superponen tres figuras de protección que tienen la misma denominación: Parque Natural del Lago de Sanabria y Alrededores, Lugar de Importancia Comunitaria (*LIC*) Lago de Sanabria y Alrededores y Zona de Especial Protección para las Aves (*ZEPA*) Lago de Sanabria y Alrededores.

El Parque Natural del Lago de Sanabria y Alrededores fue creado en el año 1978 para preservar la morfología glaciar, la flora y fauna, y la pureza de las aguas del único y mayor lago de origen glaciar de la Península Ibérica, con 318,7 ha de superficie y 53 m de profundidad. Los 227 km^2 del Parque Natural se corresponden, en su mayor parte con áreas de media y alta montaña de las Sierras Segundera y Cabrera. Desde el Moncalvo y Peña Trevinca, a más de 2.000 metros de altitud, este territorio recoge aguas de casi toda la cuenca vertiente del Lago de Sanabria y del río Tera, su principal tributario. Por debajo de la línea de las cumbres, a unos 1.700 metros de altitud, estas antiguas montañas forman una especie de altiplano ondulado cubierto de brezos y salpicado de más de una veintena de pequeñas lagunas y numerosas turberas. La escasa población del Parque se reparte entre una multitud de pequeñas poblaciones rurales pertenecientes administrativamente a cuatro ayuntamientos: Galende, Trefacio, Cobreros y Porto (Palacios Alberti & Martínez Fernández 1998).

3.1.2.1.2 Materiales litológicos y suelos

Este territorio se encuadra en la parte central del Macizo Ibérico, concretamente en el área más septentrional de la Zona Centroibérica, en el Dominio tectónico del Ollo de Sapo. Desde el punto de vista estratigráfico, este Dominio se caracteriza por presentar extensos afloramientos de rocas de porfiroides y gneises glandulares debajo de las series pizarrosas del Ordovícico Inferior (Martínez Catalán et al. 2004). Se encuentran rocas correspondientes al Paleozoico, que abarcan desde el Cámbrico hasta el Ordovícico Inferior. Los materiales mejor representados son porfiroides y gneises que corresponden a la Formación Ollo de Sapo y constituyen extensos afloramientos situados en el núcleo de los anticlinales. Son los materiales sobre los que se asientan las dos localidades estudiadas, aunque gran parte de la zona denominada como Galende se encuentra sobre depósitos fluvioglaciares, que engloban principalmente sedimentos arcillosos y limosos, con gran cantidad de micas detríticas y en algunos casos arenas de cuarzo (Figura 35). En el territorio también existen rocas graníticas y metamórficas, debidos a la Orogenia Hercínica, que afloran en una amplia extensión, tratándose de materiales que apenas están recubiertos por depósitos cuaternarios de origen glaciar (morrenas) y que presentan escaso desarrollo edáfico. Del Holoceno existen aluviones, constituidos principalmente por arenas, en la actual vega de Ribadelago.

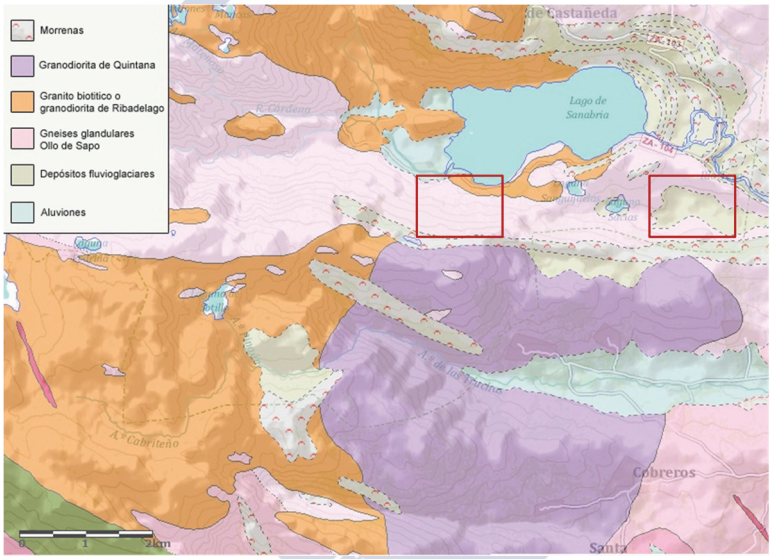


Figura 35. Materiales litológicos presentes en el área de estudio, y en las zonas muestreadas de Monte Devesa (izqda.) y Vegas de Galende (dcha.) (Fuente: Elaboración propia a partir de la cartografía del IGME).

Los suelos presentes en estas localidades son brutos y poco desarrollados, limitados por condiciones edáficas desfavorables. En general, presentan una escasa profundidad, situándose el sustrato de rocas impermeables en niveles cercanos a la superficie. Son ácidos, cuyo grado de acidez depende básicamente de la roca madre sobre la que se desarrollan. Con frecuencia, en las áreas graníticas y gnésicas aparecen extensas superficies dominadas por afloramientos rocosos, como sucede en la parte oeste de Monte Devesa; mientras que los suelos más fértiles y profundos se circunscriben a las llanuras formadas en los laterales o el frente de los antiguos glaciares, como es el caso de Vegas de Galende. Se trata de los Leptosoles úmbricos, suelos con muy escaso desarrollo, de color pardo negruzco originados sobre rocas silíceas. Tienen perfil tipo A/C con un horizonte A úmbrico muy humífero que no supera los 25 cm de espesor. El horizonte A está compuesto por abundante materia orgánica muy poco trasformada. Si la humedad del emplazamiento aumenta, como es el caso en la localidad de Vegas de Galende, tienden a formarse suelos anmooriformes, en los que el nivel del agua alcanza la superficie del suelo de manera intermitente, manteniendo sin embargo un grado de humedad muy elevado y encontrándose a veces el suelo saturado de agua. Son de aspecto negro y cenagoso y la formación

húmica se encuentra mezclada con materia mineral. En las partes de mayor pendiente de la localidad de Monte Devesa aparecen los Leptosoles líticos, limitados en profundidad por la roca continua y dura, con escaso o inexistente desarrollo.

3.1.2.1.3 Caracterización climática

La altitud y la estructura del relieve son los responsables directos de la variabilidad climática del Parque. Los vientos dominantes en este territorio son del NE, de clara influencia continental, y también del O y SO, de procedencia atlántica. Los vientos procedentes del atlántico son templados y húmedos y traen consigo las abundantes precipitaciones que afectan a la comarca. En otoño, invierno y primavera se generan importantes nevadas en las partes más altas de la sierra. En verano, cuando el anticiclón de las Azores está posicionado más al sur, se produce una intensa insolación y sequía estival en la parte más occidental sanabresa. En ocasiones, la zona recibe vientos del oeste cargados de humedad que atenúan la aridez y las altas temperaturas estivales, hecho que sin embargo no puede evitar la influencia continental. Junto a estos vientos que condicionan el clima general de la zona, existen otros debidos a las condiciones locales. En los valles, como el de Tera, se registran vientos y brisas de inversión térmica, es decir, vientos anabáticos, que suben por las laderas por el día y los vientos catabáticos que descienden desde las cumbres por la noche (Díaz García 2002).

Las precipitaciones anuales medias, sobre una muestra de 36 años, son de 1.465 mm en Ribadelago, 1.568 mm en Presa Cárdena y de 1.000 mm en Puebla de Sanabria (Tabla 2), alcanzándose cifras extremas de 2.000 mm en algunos años y de tan sólo 600 mm en otros. El 40% de estas precipitaciones se concentra en los meses de noviembre, diciembre y enero, lo que unido a un coeficiente de escorrentía de 0,78 da lugar al régimen torrencial de los arroyos en los meses de invierno con frecuentes avenidas. En cambio, las precipitaciones correspondientes a los meses de junio, julio y agosto sólo representan el 8 % del total anual, lo que se traduce en un estiaje bastante pronunciado de los cauces.

Pluviometría media mensual (mm)

Estación meteorológica	ENE	FEB	MAR	ABR	MAY	JUN	JUL	AGO	SEP	OCT	NOV	DIC	Total
Ribadelago	202	186	119	110	107	57	23	19	70	164	195	214	**1.465**
Presa Cárdena	190	167	124	122	129	80	34	27	85	191	208	213	**1.568**
Puebla de Sanabria	138	123	70	79	81	42	20	17	56	113	123	138	**1.000**

Tabla 2. Valores medios mensuales de la precipitación (mm).
(Fuente: http://www.magrama.gob.es/es/)

Las precipitaciones se producen sobre todo en otoño e invierno, las invernales son principalmente en forma de lluvia, aunque en las altitudes superiores a 1.300 m se da frecuentemente en forma de nieve o aguanieve. Los lugares más elevados reciben cuatro veces más cantidad de nieve: 41,5 días de media con nieve en Cárdena (Sierra Segundera, 1.700 m) frente a los 10,8 días de media en Puebla de Sanabria (900 m).

Temperatura media mensual (ºC)

Estación meteorológica	ENE	FEB	MAR	ABR	MAY	JUN	JUL	AGO	SEP	OCT	NOV	DIC	Media
Ribadelago	3,2	4,0	6,3	8,0	11,1	15,6	18,9	18,4	15,6	10,9	6,8	4,0	**10,2**
Presa Cárdena	-1,0	-0,9	1,2	3,0	6,7	11,1	14,7	14,4	11,5	6,6	2,7	-0,1	**5,8**
Puebla de Sanabria	2,5	3,6	5,9	7,8	11,3	15,1	17,9	17,3	14,5	10,1	5,8	3,3	**9,6**

Tabla 3. Valores medios mensuales de la temperatura (ºC).
(Fuente: http://www.magrama.gob.es/es/)

Las temperaturas medias anuales rondan los 10 ºC en Ribadelago y Puebla de Sanabria, descendiendo hasta los 6 ºC en Presa Cárdena (Tabla 3). El periodo libre de heladas se extiende desde el 15 de mayo al 15 de octubre. Los inviernos son moderadamente largos con temperaturas mínimas de hasta 11 ºC bajo cero en los meses de enero y febrero (en Presa Cárdena). En verano, julio y agosto, se alcanzan máximas próximas a los 30 ºC (en Ribadelago y Puebla de Sanabria).

3.1.2.1.4 Vegetación

En las últimas propuestas de sectorización biogeográfica (Rivas-Martínez et al. 2002) la zona de estudio está próxima al límite entre la Región Mediterránea y la Eurosiberiana, si bien queda claramente incluida en la primera. El Parque Natural del Lago de Sanabria y Alrededores se ubica en la Provincia Mediterránea Ibérica Occidental, Subprovincia Carpetano-Leonesa, Sector Berciano-Sanabriense, Subsector maragato-sanabriense. Esta situación de límite biogeográfico confiere una gran originalidad botánica a este territorio, muy emparentado con el resto las montañas y las penillanuras mediterráneas del reborde occidental de la depresión del Duero pero expuesto a las influencias climáticas y sus afinidades florísticas, con un amplio área que abarca la mayor parte de Galicia y la zona noroeste de Portugal en lo que se ha denominado Sector Galaico-Portugués, ya en la Región biogeográfica Eurosiberiana.

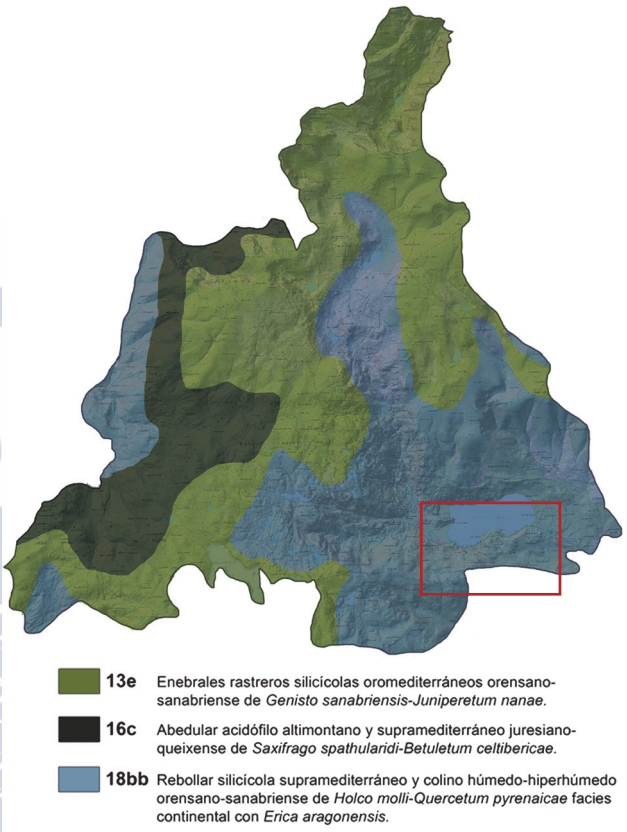


Figura 36. Mapa de vegetación potencial del Parque del Lago de Sanabria, con la localización de la zona de estudio. (Fuente: Elaboración propia a partir de Rivas-Martínez (1987)).

En el piso supramediterráneo aparecen los rebollares, que son las formaciones estudiadas y las protagonistas del paisaje vegetal de las zonas basales de este Espacio Natural (Figura 36). Estos bosques, en diferentes grados de evolución, tallas, coberturas, etc. cubren laderas y fondos de vaguada en altitudes por debajo de 1.700 m. Sus matorrales de sustitución, brezales de la asociación *Halimio ocymoidis-Ericetum umbellatae* que ocupan grandes extensiones en las montañas al exigir ombroclimas húmedos, y que surgen como consecuencia de la degradación edáfica causada por reiterados incendios, así como escobonales de la asociación *Genisto hystricis- Cytisetum multiflori*, dominan el tapiz vegetal desarbolado de este entorno. Fitosociológicamente, en ambas localidades la vegetación estudiada se corresponde con un **rebollar silicícola supramediterráneo y colino húmedo e hiperhúmedo orensano-sanabriense de *Holco molli-Quercetum pyrenaicae* facies continental con *Erica australis*** (Rivas-Martínez 1987).

En este nivel, se mantienen algunas comunidades emparentadas con series de vegetación de óptimo eurosiberiano desarrolladas al abrigo proporcionado por vertientes abruptas y circos glaciares orientados al norte. Por un lado aparecen restos de abedulares silicícolas orocantábricos (*Luzulo henriquesii-Betuletum celtibericae*) entre los 1.400 m y 1.750 m de altitud intercalados con acebales de marcado carácter serial, que en conjunto presentan una composición florística similar a la descrita en el extremo occidental cantábrico (Silva Pando 1990; Izco 1996). Es un bosque puntualmente representado (vaguadas a 1.500 m), con requerimientos de humedad edáfica y menos termófilo que el robledal, por lo que se refugia

generalmente en lugares de mayor altitud, ligado a cursos fluviales permanentes. Existen pequeños reductos de vegetación emparentados florísticamente con los hayedos naviano-ancarenses, en los que está ausente la especie característica (*Fagus sylvatica*), pero que conservan otras especies frecuentes en estos bosques como *Corylus avellana*, *Taxus baccata, Paris quadrifolia* L., etc. A media altitud (<1.100-1.150 m) se sitúa la serie climatófila subhúmeda silicícola del rebollo *Genisto falcatae-Quercetum pyrenaicae*. Dentro del dominio de esta serie se conservan representaciones puntuales de encinares silicícolas incluidos en la asociación *Genisto hystricis-Quercetum rotundifoliae*, y cambronales sobre afloramientos rocosos pizarrosos orientados a poniente, como en Peñón de Castro (Ribadelago) y en las inmediaciones de Puebla de Sanabria, de porte almohadillado (Ramil Rego et al. 2001).

A partir de los 1.700 m., en el piso oromediterráneo, el dominio de los rebollares y sus matorrales de sustitución se cede progresivamente a favor de los enebrales rastreros silicícolas oromediterráneos orensano-sanabrienses de *Genisto sanabriensis-Juniperetum nanae*, que aparecen hoy en día restringidos a espolones y áreas protegidas por neveros a causa de los incendios reiterados. Cuando desaparecen por esta causa, en su lugar aparecen matas de piornales de *Cytiseto scopario-purgantis*. En este piso bioclimático se desarrollan con profusión comunidades vegetales adaptadas a condiciones de frío intenso y un prolongado período de heladas y de innivación, destacando pastizales psicroxerófilos, pastizales hidrófilos y comunidades de turberas.

3.1.2.1.5 Localidades muestreadas

Las localidades muestreadas fueron seleccionadas entre los rebollares existentes a lo largo del Parque Natural, aquellas donde el rebollo constituía masas más o menos extensas, teniendo en cuenta su posición dentro de un gradiente altitudinal así como la presencia de individuos aptos para estudios dendrocronológicos, descartándose aquellas zonas donde el roble presentaba porte arbustivo o de escasa edad. Son masas dominadas por *Quercus pyrenaica* procedentes de regeneración natural, en las que el grado de perturbación, la estructura del bosque y la composición florística difieren sensiblemente.

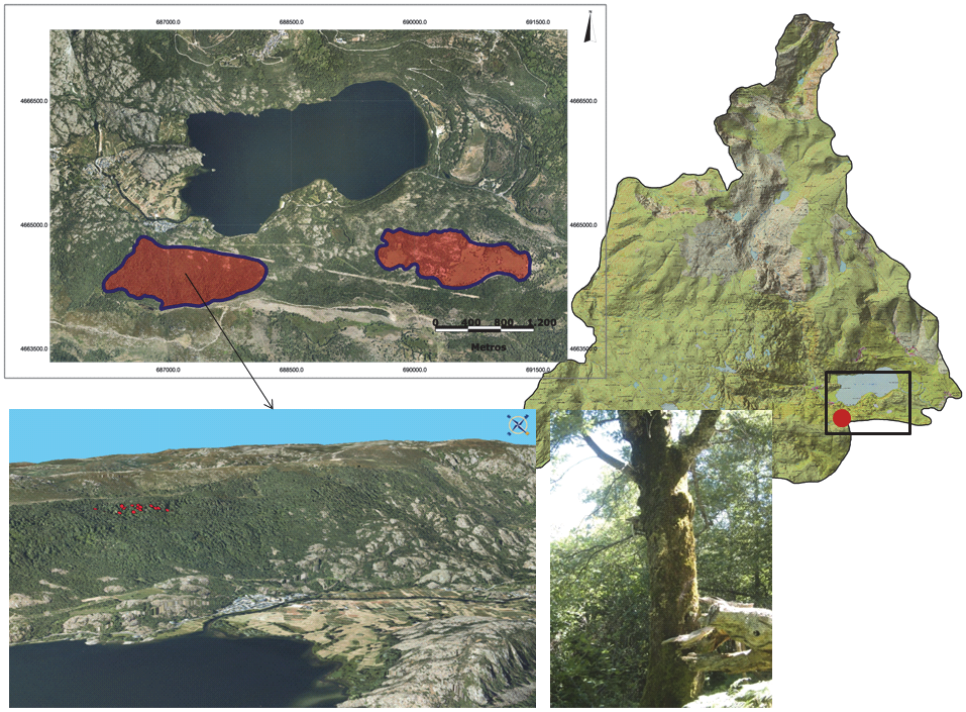


Figura 37. Masa de Monte Devesa (RIB). En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados en color rojo.

Monte Devesa (RIB)

Esta masa se encuentra al sur del lago y se sitúa principalmente en el municipio de Ribadelago (Figura 37). Se trata de un bosque mixto caducifolio, claramente dominado por *Quercus pyrenaica*, con presencia de pies de grandes proporciones tanto en porte como en diámetro. Se enclava en las proximidades de la Laguna de Carros, que constituye la parte superior de la ladera. En las partes más húmedas hay una gran abundancia de *Corylus avellana* e *Ilex aquifolium*, en las vaguadas suele desaparecer el rebollo para formarse galerías impenetrables de acebo y avellano junto a *Sorbus aucuparia, Salix atrocinerea*, etc.

En este bosque destaca una franja donde aparecen bosquetes de *Prunus padus* L., árbol raro en el NO de la Península, y propio de bosques muy húmedos, así como la presencia puntual de ejemplares de *Quercus robur*, algunos de gran talla. Es una zona de difícil acceso con fuertes pendientes y afloramientos rocosos. Los árboles estudiados provienen de la parte superior de la ladera, ya que es donde se conservan los árboles de mayor tamaño, disminuyendo notablemente la edad en las partes inferiores. En estas zonas los individuos no suelen superar los 100 años. El muestreo en esta localidad no fue fácil, ya que requirió un amplio recorrido del mismo con el fin de localizar los árboles más adecuados, que se vio dificultado por la elevada pendiente y la amplia extensión de la masa.

Vegas de Galende (GAL)

Se trata de una masa monoespecífica de rebollo situada en el fondo de un valle de morfología típicamente glaciar (Figura 38). En las laderas de pendiente moderada a suave y próximos a la cumbre, se encuentran pies jóvenes de rebrote de cepa y raíz formando rodales impenetrables de bajo porte sin apenas sotobosque. Puntualmente aparece algún árbol de mayor talla, sobre todo hacia la parte inferior de la ladera.

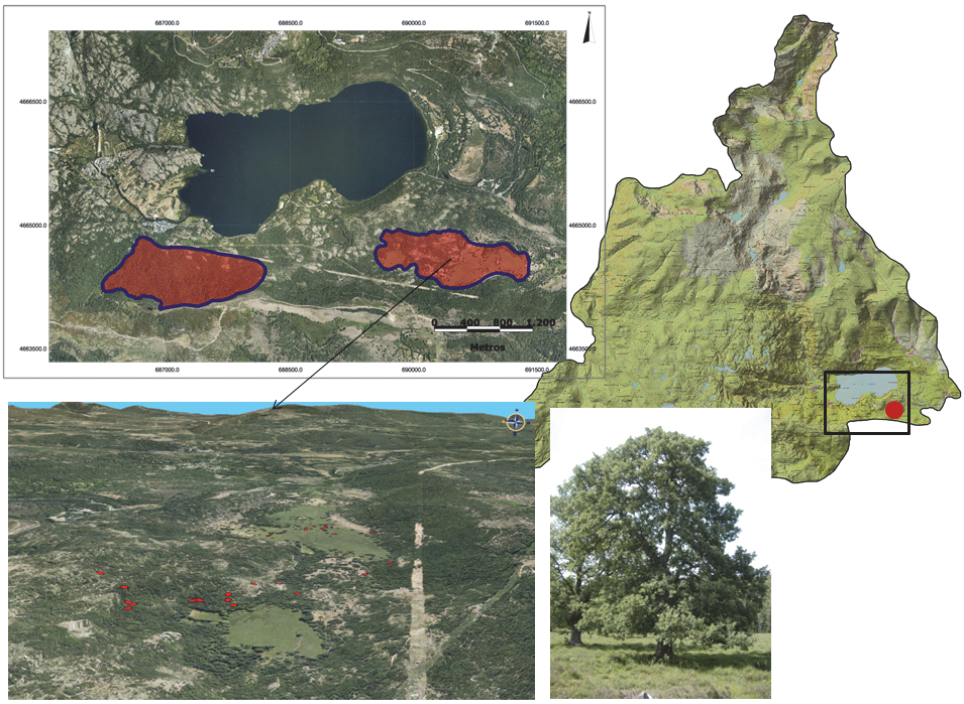


Figura 38. Masa de Vegas de Galende (GAL). En el Modelo Digital del Terreno (MDT) se pueden observar los árboles muestreados en color rojo.

Los ejemplares de mayores dimensiones y edad se encuentran a medida que se desciende la ladera y en el centro del valle, una zona muy extensa con procesos de hidroturbación sobre depósitos fluvioglaciares, donde los suelos se presentan muy ricos en materia orgánica. Esta parte inferior presenta arboles aislados entre sí, pero de edades avanzadas, llegando algunos a los 300-400 años, que han sido los seleccionados para el muestreo. Finalmente, existen notables signos de perturbación en esta localidad, ya que los árboles muestran numerosos signos de haber sido sometidos a podas, probablemente con fines de extracción de leña. El pastoreo ha sido también una práctica habitual en esta localidad.

3.2 OBTENCIÓN Y PREPARACIÓN DEL MATERIAL

3.2.1 Caracterización de las localidades y selección de individuos

En cada una de las localidades de muestreo se tomaron varios parámetros que caracterizan el medio físico, tales como ubicación geográfica, altitud, pendiente, exposición o materiales litológicos. El estudio del medio físico y de las comunidades vegetales existentes permitió seleccionar, en cada caso, un área homogénea sobre la cual se realizó el muestreo siguiendo las indicaciones de Schweingruber (1989).

La selección de los árboles objeto de muestreo se realizó siguiendo las recomendaciones habituales para la realización de estudios dendroclimatológicos, es decir, eligiendo subjetivamente aquellos individuos que, en función de la experiencia, parecen reflejar mejor las condiciones climáticas que se pretenden estudiar (LaMarche 1982). En este caso el empleo del muestreo aleatorio o sistemático carece de sentido, ya que sólo resulta adecuado para el estudio de factores no climáticos y generalmente no permite obtener suficiente número de árboles válidos para fines dendroclimatológicos (Schweingruber et al. 1992). Además, se rechazaron los ejemplares que presentaban características no deseables como efectos fuertes de competencia, daños mecánicos, ataques de hongos o insectos, etc., en los cuales el efecto climático podría no estar bien representado en los anillos.

Por lo tanto, se seleccionaron individuos aparentemente de edad avanzada (los de mayor diámetro y altura), es decir, con edad suficiente para poder prolongar la cronología lo máximo posible en el tiempo; e individuos dominantes y codominantes, ya que es de esperar que éstos reflejen mejor el efecto del clima por estar menos condicionados en su desarrollo por la competencia. En algunos casos, como el de Cabanavella, estos individuos estaban relativamente aislados, de modo que el efecto de una posible competencia con otros árboles era mínimo. Siempre se eligieron pies sanos en apariencia y sin evidencia de perturbaciones y se han incluido además algunos árboles jóvenes en las cronologías porque los pies viejos de roble a veces presentan fuertes reducciones de crecimiento, que se manifiestan con la casi total ausencia de madera tardía en los anillos y muy poca variación en su anchura (Eckstein & Schmidt 1974). Por esta razón el muestreo de árboles de diferentes edades resulta conveniente para conseguir cronologías sensibles y homogéneas (Schweingruber et al. 1992). Además, el empleo de distintas clases de edad favorece la sincronización de los árboles más viejos y aumenta la calidad de la parte final de las cronologías (Shiyatov 1986).

3.2.2 Obtención y preparación de las muestras

De los árboles seleccionados en cada localidad se extrajeron testigos de madera o *cores* de 5 mm de diámetro, utilizando una barrena manual Pressler de 40 cm de longitud (Figura 39). Debido a la dureza de la madera, no fue posible el empleo de una barrena de mayor diámetro, como sería deseable para el estudio de vasos, de manera que fue necesario disponer de al menos dos *cores* por árbol. Este hecho es importante, ya que el error de la cronología disminuye al aumentar el tamaño muestral de *cores* o de árboles, y además siempre se requiere más de un *core* para calcular diversos estadísticos (Fritts 1976), como la correlación media dentro de árboles. Por otro lado, un segundo *core* favorece notablemente la sincronización y es imprescindible para obtener un número suficientemente representativo de vasos.

Las distintas extracciones se realizaron a la altura normal (1,30 m) siguiendo las recomendaciones habituales para estudios dendroclimáticos (Fritts 1976), generalmente en posiciones opuestas del tronco y en dirección perpendicular a la pendiente del terreno con el fin de evitar la madera de tensión (Schweingruber et al. 1992). No obstante, en ciertos casos fue necesario obtener las muestras en otras partes del tronco debido a impedimentos de tipo físico como heridas, podredumbres o presencia de ramas bajas, por lo que no siempre fue posible cumplir las anteriores premisas. Este hecho no afecta negativamente a la calidad de estas muestras ya que, según Krause (1992), no existen

grandes diferencias en la variabilidad interanual de crecimiento entre diferentes partes del tronco del árbol.

El número de árboles muestreados osciló entre 14 y 45 árboles; que en ambos casos se encuentran dentro de la cantidad recomendada por diversos autores para estudios dendrocronológicos (Fritts 1976; Schweingruber et al. 1992), y las muestras obtenidas se transportaron al laboratorio introducidas en pajillas o cánulas de papel debidamente etiquetadas con el código identificativo del *core* correspondiente (tres siglas referentes a la localidad, dos cifras numéricas referentes al árbol muestreado y una letra para cada *core* extraído, p.e.: GUD09A). Estas pajillas cumplen la doble función de proteger la muestra durante el transporte y evitar su curvatura durante el secado, ya que aportan la rigidez necesaria sin dificultar la natural pérdida progresiva de humedad debido a la porosidad del papel. No se aplicó ningún tratamiento para taponar los agujeros del taladro, pues el daño producido es mínimo y únicamente se aprecia una pérdida de la coloración de la madera en el entorno de la herida, la cual se cierra totalmente al cabo de 1-3 años (Dujesiefken et al. 1989). Por otro lado, cualquier taponamiento sólo actuaría como barrera sobre la cual se vería obligado a crecer el labio de cicatrización (Schweingruber 1996).

Figura 39. Proceso de preparación de los *cores* extraídos y muestra resultante, previamente a su tinción

Durante la recogida de muestras también se anotaron en el campo una serie de parámetros de los árboles muestreados, tales como diámetro a la altura de 1,30 m, disposición de la copa, coordenadas UTM, y todas aquellas características de cada individuo que podrían resultar de utilidad en fases posteriores, como anomalías presentes en el fuste o ramas, daños físicos, etc., con el fin poder interpretar posibles patrones anormales en el crecimiento de algunos de los ejemplares. Se observó el aspecto de los *cores* recién extraídos en el campo para determinar si éstos presentaban defectos o pudriciones con el objeto de obtener al menos dos testigos apropiados por árbol, y se tomaron dos fotografías de cada individuo muestreado.

En el laboratorio, los *cores* se secaron al aire dentro de las propias pajillas o cánulas de papel durante un mínimo de 48 horas y posteriormente se montaron sobre soportes rígidos de madera

provistos de una hendidura longitudinal, fijándolos con cola no soluble en agua (Figura 39). Después de 24 horas de secado del adhesivo, se rebajó su superficie con un micrótomo de deslizamiento y se llevó a cabo un lijado en tres etapas, utilizando papel de grano progresivamente más fino (P220, P600 y P1200), hasta conseguir una superficie perfectamente pulida con una óptima visualización de los anillos de crecimiento.

Con el fin de eliminar las frecuentes tilosis y el serrín acumulado en el interior de los vasos durante la fase de lijado, se aplicó agua a presión con una hidrolimpiadora (Fonti et al. 2002), lo que conlleva ciertos riesgos si no se es especialmente cuidadoso en su utilización. Por un lado es necesario ajustar correctamente la distancia de trabajo, ya que se pueden ocasionar roturas de las paredes de los vasos, sobre todo en anillos con una gran desidad de poros o en áreas de fuertes reducciones de crecimiento. Por otro lado, las fisuras y roturas de la madera se acentúan con la rehidratación y secado de la muestra. Por tanto, es conveniente proceder con cautela durante las fases de extracción, montaje y rebajado del *core* para obtener muestras libres de roturas y minimizar así estos problemas. De esta manera los vasos quedaron perfectamente limpios y huecos.

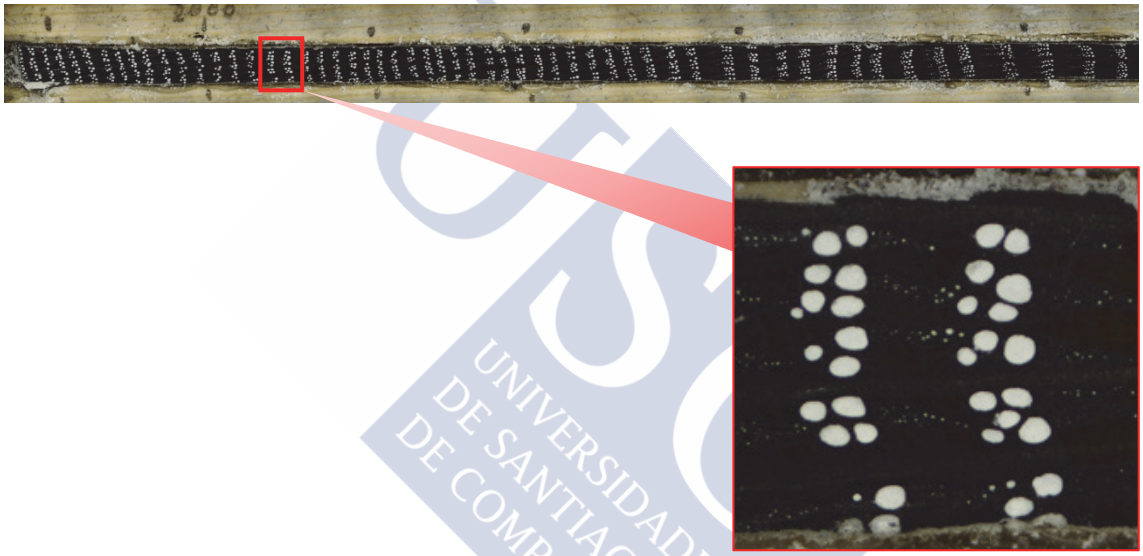


Figura 40. Aspecto de las muestras tras el proceso de tinción y contraste conseguido una vez concluidas todas las etapas de preparación

Transcurridas 24 horas de secado, se tiñeron todas las muestras con tinta negra de impresora aplicada mediante un pincel con el objetivo de uniformizar el color de la madera, y tras 2-3 horas una vez seca la tinta, se procedió a aplicar polvo de tiza sobre la superficie para rellenar los vasos (Schweingruber 1989). De esta manera se consiguió aumentar mucho el contraste entre madera y vasos (Figura 40), resaltando estos últimos y optimizando de manera muy notable el reconocimiento automático de los elementos conductores. Una buena imagen de partida es fundamental para facilitar las posteriores fases del análisis.

Las muestras así tratadas se utilizaron para la datación preliminar de los anillos, la medición de las anchuras (total, madera temprana y madera tardía), y la medición de diferentes parámetros anatómicos derivados de los vasos. Además, este tipo de preparación facilitó la selección de las muestras para la fase de análisis de imagen, al ofrecer una mejor y más contrastada visualización de anillos y vasos.

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3.3 OBTENCIÓN DE DATOS

3.3.1 Medición de la anchura de los anillos y sincronización

La medición de la anchura de los anillos de crecimiento se llevó a cabo discriminando entre madera temprana y tardía con una precisión de un micrómetro. Su diferenciación se realizó atendiendo al tamaño de los vasos, claramente más grandes en la madera temprana, y atendiendo a la estructura del tejido. En la madera temprana, los vasos aparecen de mayor tamaño, muy próximos entre sí y formando una banda continua. Sin embargo, en la madera tardía estos vasos son más pequeños y tienden a formar grupos alargados y engrosados hacia el extremo final. Este criterio fue empleado con éxito por otros autores para el roble (García-González & Eckstein 2003; González-González et al. 2014).

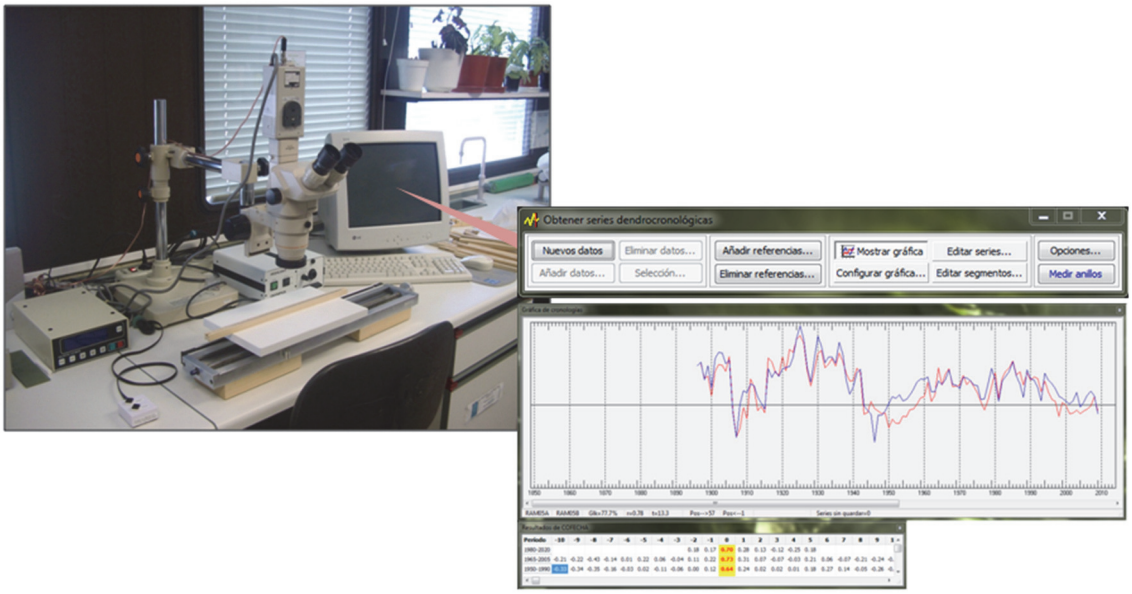

Figura 41. Medidor VELMEX y software utilizado para la medida de las anchuras de los anillos de crecimiento.

La anchura de los anillos se midió siempre perpendicularmente a su límite, siguiendo la dirección marcada por los radios (Stokes & Smiley 1968). Para ello se usó una lupa binocular, habitualmente bajo aumentos de 20-40x, dotada de una cruz filar de referencia y complementada con un medidor de anillos modelo *Velmex* (Figura 41). El medidor a su vez está integrado con un software específico *DendroGMT* (García gonzález, no publicado) cuya versatilidad permite, además del almacenamiento de las medidas, su representación gráfica y tratamiento posterior para llevar a cabo la sincronización de las series y la corrección de errores.

El proceso de sincronización consiste básicamente en asignar a cada uno de los anillos de crecimiento el año exacto de su formación (Schweingruber 1989). Para llevarlo a cabo se compararon visualmente las distintas curvas de crecimiento de las series de anchura total de los anillos, tratando de buscar una variación interanual similar (Figura 41), con lo que se logra corregir los errores cometidos durante la medición, aclarar segmentos o anillos dudosos y localizar anillos falsos o ausencias locales de éstos. Dicha comparación se complementó con diversos tests estadísticos para verificar la correcta sincronización, usando el programa *Cofecha* (Grissino-Mayer 2001) y diferentes rutinas de programación desarrolladas por I. García González. No fue necesario repetir el proceso de sincronización para la madera temprana y tardía, ya que se trabajó sobre un mismo conjunto de datos, de modo que los errores de medición eran corregidos para las tres variables, como se suele hacer cuando se trabaja con variables anatómicas (García-González & Eckstein 2003; Fonti & García-González 2004). No obstante, las

curvas de anchuras del leño temprano y tardío también fueron analizadas para localizar la presencia de valores extremos que pudiesen ser debidos a errores de medida.

Esta es la fase más importante y laboriosa de los estudios dendrocronológicos y sin duda alguna constituye un proceso imprescindible, ya que es el punto de partida de las fases posteriores y es el proceso del que depende en gran medida la calidad de la cronología obtenida.

3.3.2 Análisis de la anatomía de la madera

3.3.2.1 Selección de muestras

En cada una de las localidades se seleccionó, en base al material recogido, una submuestra para llevar a cabo el análisis de imagen. Se escogieron solamente aquellos árboles cuyos *cores* no presentasen podredumbres, roturas o anomalías de la madera que dificultasen su estudio, y cuyas series de anchuras de crecimiento presentaran una alta correlación estadística con la cronología local. Se empleó un total de dos *cores* de 5 mm de anchura por cada árbol seleccionado, número en general suficiente para que los resultados sean representativos, siendo sólo ligeramente inferior a la anchura tangencial necesaria para lograr una óptima representatividad (Fonti & García-González 2008).

El número de árboles muestreados y seleccionados para análisis de imagen en cada localidad, así como el periodo temporal que cubren, pueden consultarse en la Tabla 4.

Localidad	*Código*	*Especie*	*Nº Árboles muestreados*	*Nº Árboles para Análisis de imagen*	*Período cubierto*
Fraga dos Casás	**MER**	*Q. robur*	22	14	1936-2012
Vilapena	**VIP**	*Q. robur*	24	12	1938-2007
Estornín	**EST**	*Q. robur*	25	12	1908-2007
Cabanavella	**CAB**	*Q. robur*	45	24	1520-2003
Ramirás	**RAM**	*Q. robur*	14	12	1890-2013
Vilanova dos Infantes	**INF**	*Q. robur*	15	13	1840-2013
Villaoscura	**VIO**	*Q. pyrenaica*	18	10	1915-2012
Sober	**SOB**	*Q. pyrenaica*	15	10	1885-2012
Castro Caldelas	**CAL**	*Q. pyrenaica*	20	12	1904-2012
Fradelo	**FRA**	*Q. pyrenaica*	19	12	1929-2012
A Gudiña	**GUD**	*Q. pyrenaica*	20	13	1926-2012
Galende	**GAL**	*Q. pyrenaica*	28	10	1937-2002
Monte Devesa	**RIB**	*Q. pyrenaica*	17	14	1937-2002

Tabla 4. Número de árboles totales muestreados y submuestra para análisis de imagen, en cada una de las localidades de estudio.

3.3.2.2 Análisis de imagen

El término análisis de imagen hace referencia al estudio realizado sobre una imagen con el fin de obtener información de ella (Pertusa Grau 2003). Consta habitualmente de tres fases secuenciales y complementarias: (1) la **captura**, que constituye un paso intermedio entre la realidad y la percepción humana de la misma; (2) el **procesado o manipulación**, que consiste en la elaboración de una imagen para producir otra que resalte los parámetros a medir o analizar; y (3) el **análisis** propiamente dicho, proceso por el cual se extrae información cuantitativa de dicha imagen. En dendrocronología, el análisis de imagen se emplea sobre todo para obtener series de datos relativos a las dimensiones o densidades de los elementos conductores (García-González & Fonti 2008; Gea-Izquierdo et al. 2012; González-González et al. 2014) mediante su diferenciación del tejido xilemático restante, siendo empleados para la elaboración de series temporales de los parámetros de interés.

3.3.2.2.1 Captura de las imágenes de los anillos de crecimiento

Después de haber verificado la correcta datación de los anillos en los *cores* seleccionados para cada localidad, y como paso previo a la captura de imágenes, se realizó un cuidadoso repaso de la superficie de cada una de las muestras bajo la lupa binocular. Así se consiguió la detección y corrección temprana de ciertas deficiencias, como la existencia de vasos que pudieran haber quedado sin rellenar con el polvo de tiza o de áreas emborronadas y poco nítidas, que entorpecerían en alguna medida las fases posteriores. En los casos de muestras defectuosas, se corrigieron estos defectos mediante el repulido, la nueva tinción y el tratamiento con tiza hasta que las muestras quedaron en perfecto estado para la captura de las imágenes.

Figura 42. Equipo de fotografía ubicado en las instalaciones del IBADER, donde se pueden observar los distintos componentes.

Para la captura de imágenes se utilizaron dos procedimientos diferentes. En el primer procedimiento, parte de las muestras estudiadas en esta tesis se fotografiaron utilizando un equipo de fotografía disponible en el IBADER (Instituto de Biodiversidad Agraria y Desarrollo Rural) (Figura 42), compuesto por una cámara digital modelo Nikon DMX 1200F montada sobre un estereomicroscopio óptico binocular modelo Nikon Eclipse 90i, un ordenador al que está conectada dicha cámara y un software específico de Nikon. Las fotografías se tomaron manteniendo la dirección paralela a los radios con la ayuda de una plataforma móvil que, accionada manualmente, desplaza la muestra a intervalos más o menos regulares, permitiendo capturar las imágenes con cierto grado de solapamiento, variable de unas imágenes a otras. Para obtener una iluminación uniforme de toda la muestra, el microscopio cuenta con un anillo LED de luz fría regulable en intensidad, por lo que se pudo adaptar la cantidad de luz necesaria a cada caso particular.

La utilización de este equipo supuso una considerable inversión de tiempo y, pese a la gran calidad de las imágenes obtenidas, fueron relativamente frecuentes algunos errores derivados del manejo manual de la plataforma. Así, la irregularidad del porcentaje de solapamiento entre fotografías hizo que, en ocasiones, resultara insuficiente para la posterior fusión de imágenes. En otros casos los errores fueron debidos a la omisión o repetición de uno o varios anillos en zonas de extensas y acentuadas reducciones del crecimiento. La detección y corrección de estos problemas dificultaron las fases posteriores del análisis, haciendo necesario repetir algunas de las fotografías con el consiguiente retraso ocasionado.

Para evitar estos errores y agilizar en la medida de lo posible la digitalización de muestras, se utilizó un segundo procedimiento tras el desarrollo recientemente de un aparato llamado **SIFAU**

(SIstema de **F**otografía **AU**tomático**)** (García González & Souto Herrero, no publicado) con el que se fotografiaron de forma automática el resto de las muestras de esta tesis. Dicho dispositivo integra un equipo de microfotografía (Lupa Olympus SZ60 + Anillo de iluminación LED + Cámara fotográfica Canon EOS 600D), una plataforma móvil Lintab accionada por un servomotor de giro continuo conectado a un circuito con salida a puerto USB, y un software informático específico, denominado **ServoFotos** y desarrollado por I. García González, que controla el funcionamiento del conjunto (Figura 43).

Figura 43. Sistema de Fotografía Automático (SIFAU).

De esta manera, ajustando en el programa parámetros como el porcentaje de solapamiento entre imágenes y la longitud del *core*, éste calcula las fotografías necesarias y activa automáticamente el movimiento de la plataforma y el disparo de la cámara. Con estos ajustes se optimiza el número de imágenes necesarias para abarcar la totalidad del *core*, evitando el consumo innecesario de recursos, tanto de almacenaje en soporte informático como de Memoria RAM por parte del software de fusión empleado en la siguiente fase del trabajo. Ambos equipos trabajan perfectamente sincronizados por *ServoFotos*, que además accede al software propio de la cámara (EOS Utility v. 2.10.2.0), donde se realizan ajustes tales como apertura del diafragma, tiempo de exposición u opciones de guardado de las fotos resultantes, entre otros.

Dado el volumen de muestras a analizar (346 *cores* procedentes de los 168 árboles seleccionados en total), el desarrollo y utilización de este aparato supuso un notable ahorro de tiempo y esfuerzo en esta fase del trabajo. Además, al automatizarse las tareas se redujeron en gran medida los errores descritos anteriormente y, de cometerse alguno, la captura de la secuencia completa de imágenes de un testigo no supondría apenas retraso debido a la gran rapidez con la que trabaja SIFAU.

Las fotografías tomadas tanto con el equipo del IBADER como con SIFAU presentaron una alta resolución, un factor decisivo ya que ésta condiciona en gran medida la precisión en la manipulación y análisis posteriores (Pertusa Grau 2003). De esta manera, se pudieron obtener óptimos resultados en el empleo de las técnicas de análisis de imagen, que son dependientes de la calidad de captura (Spiecker 2000). Con ambos equipos, cada una de las muestras fue digitalizada inicialmente en una serie de imágenes individuales correspondientes a porciones consecutivas de la misma, existiendo un porcentaje de solapamiento entre imágenes contiguas. Este porcentaje de solapamiento o zona común entre fotografías, como ya se ha mencionado anteriormente, pudo ser ajustado a un valor óptimo y constante del 40% en el caso de SIFAU, mientras que en el caso del equipo de fotografía del IBADER dicho valor fue variable debido a que el trabajo se desarrolló de forma manual. Las imágenes individuales así obtenidas se fusionaron posteriormente con un software específico de fusión de imágenes, PTGui Pro v.9.1.8, resultando una única imagen para cada uno de los *cores* (Figura 44). Todas ellas se salvaron en formato Tagged Image File Format (TIFF), de modo que no existiese pérdida de calidad en las mismas. Se trabajó con el mismo formato en las fases posteriores del análisis.

Debido a la existencia de una limitación de tamaño de 2^{15} (32.768) píxeles en sentido horizontal, para la correcta visualización de las imágenes por parte de algunos de los programas utilizados en las siguientes fases del trabajo, fue necesario fragmentar nuevamente la imagen resultante para cada muestra en 2-4 segmentos, de manera que ninguno excediera de aproximadamente 30.000 píxeles en alguno de sus ejes. Este proceso fue llevado a cabo con el mismo programa de fusión mencionado anteriormente, ya que permite cargar todas las imágenes individuales, componer la fotografía completa del *core* y salvar únicamente un área de selección del tamaño adecuado de una manera sencilla, rápida y exacta. Cada imagen se clasificó con un código compuesto por el código del *core* al que pertenecen seguido de un número correlativo de dos cifras separado del anterior por un guión (ej.: GUD09A-01). De esta manera las imágenes finales fueron correctamente etiquetadas, facilitando su seguimiento durante cada una de las fases del trabajo.

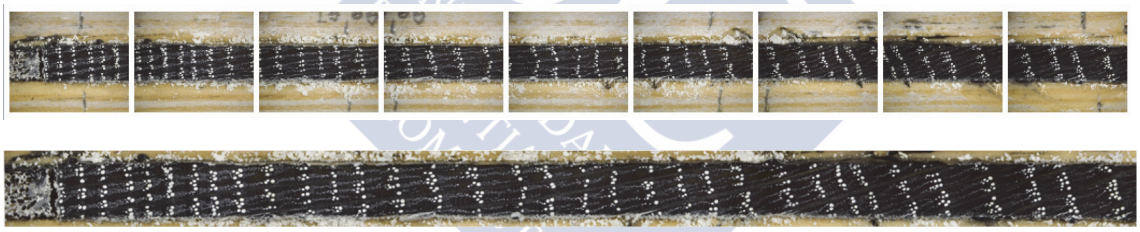

Figura 44. Imágenes individuales de una de las muestras analizadas (arriba) y resultante del proceso de fusión (abajo), esta última destinada a tratamiento digital y análisis de parámetros anatómicos.

3.3.2.2.2 Procesado y Análisis de cada imagen

Las imágenes resultantes de la fase anterior fueron analizadas con *VesselJ*, un plugin desarrollado específicamente para el análisis de elementos conductores por I. García González (no publicado). Se diseñó como un complemento del procesador de imágenes ImageJ (software libre y gratuito basado en el lenguaje de programación Java). Este programa cuenta con gran aceptación en el mundo científico (Abramoff et al. 2004) y dicho plugin (*VesselJ*) ya ha sido utilizado con éxito por Gómez Peñate (2011) y Mariño Méndez (2014)para el análisis de los vasos primaverales de diferentes especies de roble.

Los vasos de la madera temprana fueron reconocidos y analizados mediante dicho software, que permite diferenciar los objetos que se quieren analizar (vasos) del fondo (tejido xilemático y otros objetos detectados) en base a la intensidad de color de la imagen (Figura 45). Para ello es fundamental conseguir un buen contraste entre ambos, logrado mediante la tinción del tejido xilemático fundamental y la posterior aplicación del polvo de tiza al lumen de los vasos.

Aunque las imágenes obtenidas hasta este punto fueron en color, el análisis se realizó en todos los casos sobre imágenes de 256 niveles de gris (8 bits), ya que facilitan su observación y permiten una detección igualmente precisa de los elementos conductores pero con un menor consumo de recursos del ordenador. La transformación de imágenes en color a 8 bits se realizó por el método habitual en ImageJ, que utiliza diferentes porcentajes para cada uno de lo canales de color. Así, el lumen de los vasos pudo ser diferenciado del tejido restante en función del nivel de gris.

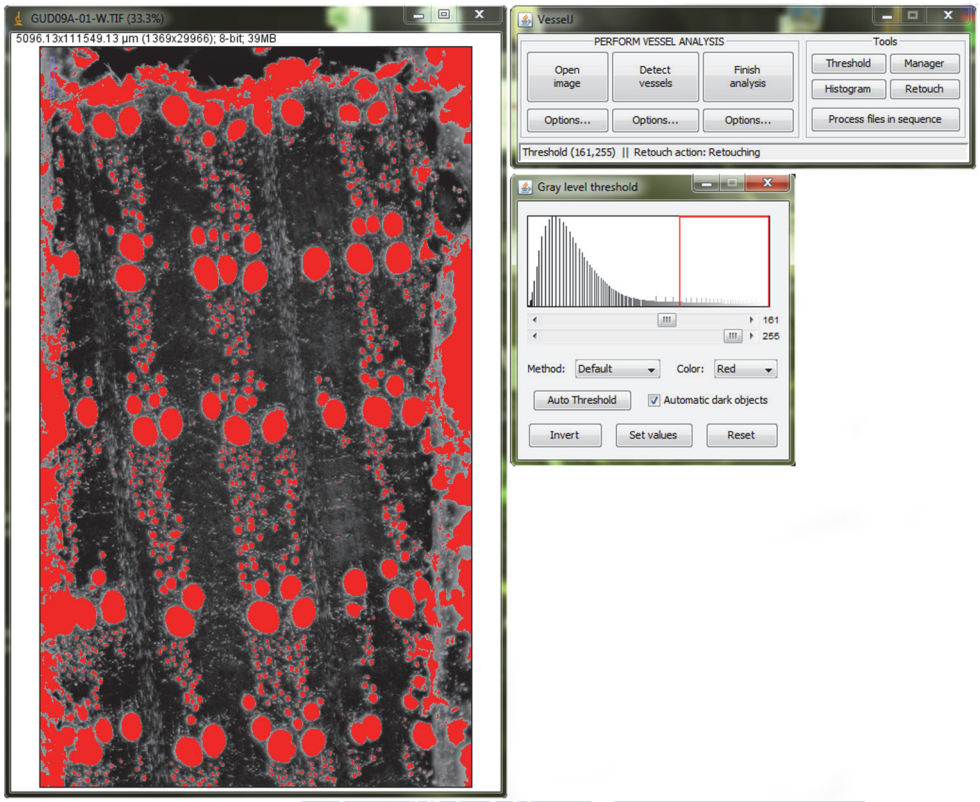

Figura 45. Discriminación por contraste entre los objetos a analizar (vasos) y el resto del tejido de la muestra, utilizando VesselJ.

Para evitar que el sistema reconociera partes de los radios leñosos, roturas, fisuras de la madera, etc. como elementos a analizar, así como vasos de menor tamaño no pertenecientes a la madera temprana, fue necesario aplicar dos filtros sobre los valores obtenidos antes de almacenar los datos resultantes:

- Filtro de tamaño, fijando un valor mínimo de 10.000 μm^2, ya que hay autores que consideran que el tamaño mínimo para clasificar a los vasos como pertenecientes a la madera temprana se sitúa entre 5000 y 10.000 μm^2 (García-González & Eckstein 2003; Fonti & García-González 2004; Eilmann et al. 2006), si bien la elección de un límite inferior relativamente alto tiene poca influencia sobre los resultados finales (García-González & Fonti 2006).

- Filtro de forma, basado en la circularidad de los objetos detectados, entendiendo ésta como la proporción entre los diámetros máximo y mínimo. Se consideró que un objeto era excesivamente alargado si este valor era inferior a 0,5, permitiendo así excluir objetos como grietas, roturas o restos de polvo de tiza que pudieran ser detectados como vasos.

Además de estos filtros, durante la realización de las medidas se aplicaron otros que actúan directamente sobre la imagen. Se aplicó una operación morfológica de erosión-dilatación (2 × 2 en un

paso), que elimina el contacto entre objetos muy próximos, y se calculó el perímetro convexo, optimizando con ambos el contorno de los vasos.

Pese a la cuidadosa preparación de las muestras y al empleo de estos filtros, en ciertas ocasiones no resultaron suficientes para eliminar todos los objetos indeseados u obtener medidas precisas, siendo necesario recurrir entonces al proceso de retoque manual de las imágenes (Figura 46).

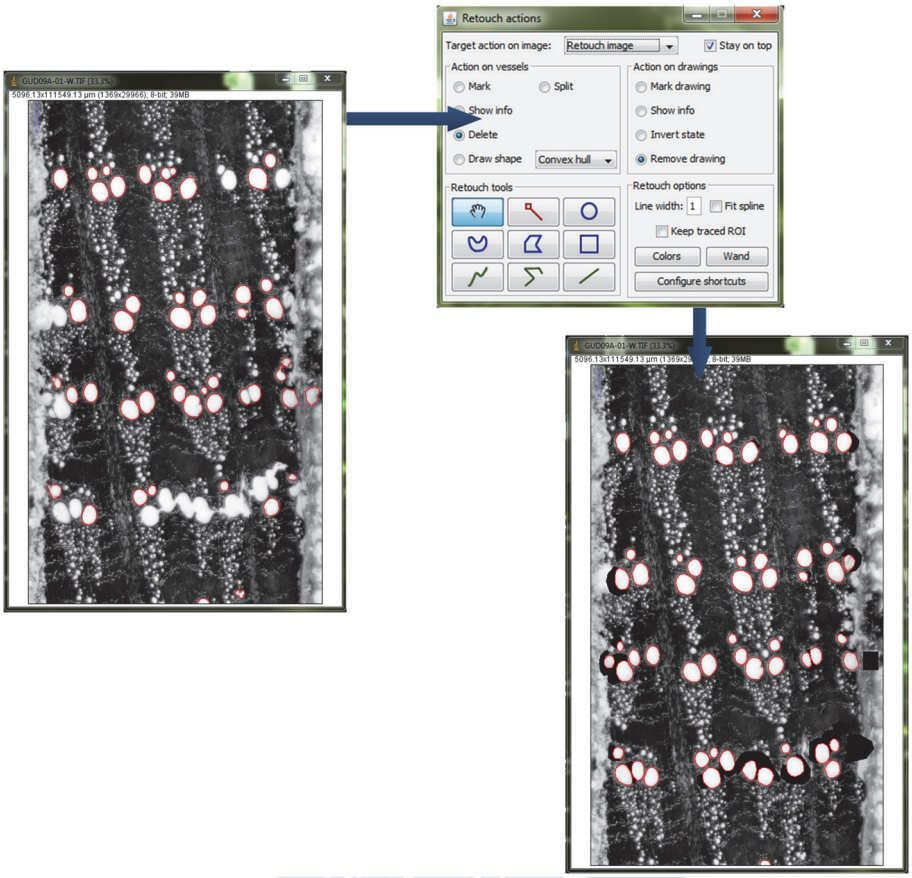

Figura 46. Fragmento de una de las imágenes analizadas, antes y después del proceso de retoque manual.

Una vez finalizado el análisis de cada una de las imágenes, *VesselJ* guarda la información referente a todos y cada uno de los vasos (área, centroide, rectángulo que lo encierra, elipse que lo encierra, diámetros, etc.) en un mismo fichero **.zip* con el nombre de la imagen. La reasignación de cada vaso (y de la información asociada) al anillo de origen o datación, fue realizada con la ayuda de otra herramienta informática llamada **AutoVasos** (Figura 47). Se trata de un software específico para especies de anillo poroso desarrollado por I. García González (no publicado) y que fue utilizado por vez primera por Gómez Peñate (2011). Este programa permite asignar a cada anillo su año correcto de formación trabajando directamente sobre la imagen original, a la cual le agrega los contornos de cada uno de los vasos detectados y los límites entre anillos consecutivos. A partir de una datación automática previa, en la que también hace una preselección de vasos de la primera hilera, el usuario puede variar manualmente tanto los elementos conductores que forman parte de dicha hilera como la afiliación de los distintos vasos a un año determinado, ajustando así la sincronización. La versatilidad de este programa permite, además, llevar a cabo correcciones posteriores de manera rápida y sencilla.

Se guardó por separado la información relativa a los vasos que conforman la primera hilera de madera temprana (Figura 47) porque se ha comprobado que en ocasiones pueden aportar información adicional y de interés (García-González & Fonti 2006; Fonti et al. 2007; González-González et al. 2014).

El criterio objetivo adoptado para establecer si un vaso era de la primera hilera fue cuando éste se situaba sobre la línea imaginaria que atravesaría la zona central de los primeros vasos formados. No obstante, debido a la experiencia acumulada por el autor de esta tesis y por el grupo de investigación, en muchos casos la distribución de los elementos conductores en el anillo permitió decidir sin dudas si un vaso era incluido o no. Actualmente se están desarrollando trabajos de xilogénesis en el grupo de investigación que apoyan en gran medida estas decisiones.

Por último, con la ayuda de una rutina de programación diseñada por I. García González (no publicado), se verificó cada una de las dataciones llevadas a cabo con AutoVasos. Para ello, se compararon las curvas de crecimiento de la anchura total, previamente sincronizadas, con las curvas de anchura resultantes del proceso anteriormente descrito (Figura 47).

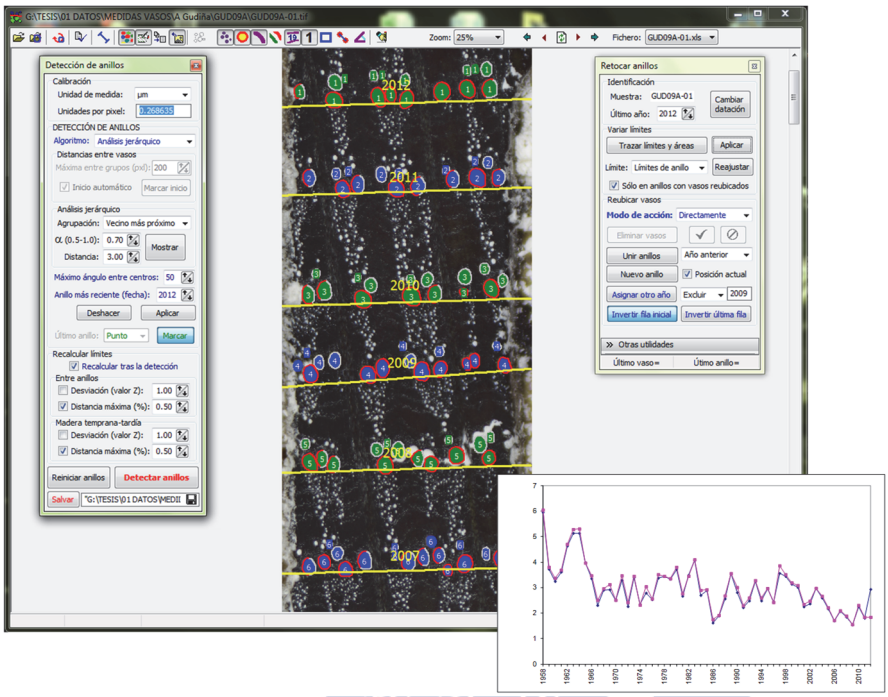

Figura 47. Programa AutoVasos, donde se pueden apreciar los diferentes anillos, los vasos pertenecientes a cada uno de ellos y los seleccionados como pertenecientes a la primera hilera formada (contorno rojo). A la derecha, verificación gráfica de la correcta datación de los vasos mediante las curvas de crecimiento.

3.3.2.2.3 Variables de crecimiento obtenidas

Preliminarmente se obtuvieron 57 variables derivadas de los vasos: Número de vasos, densidad, áreas media, máxima y total, conductividades potencial y específica, diámetro hidráulicamente ponderado, 7 percentiles de tamaño entre el 5 % y el 95 % y los 3 cuartiles, calculadas tanto para el total de vasos como para la primera fila y el resto de vasos de la madera temprana por separado. El número de vasos, el área total y la conductividad potencial también se obtuvieron para la proporción de vasos de la primera fila respecto al total del anillo.

Debido a la altísima relación existente entre muchas de ellas, que indica una gran similitud en la información aportada, se seleccionaron solamente 12 para llevar a cabo todos los análisis de la Tesis, además de las anchuras del anillo (Tabla 5). Pese a que se midieron varios parámetros en cada uno de los vasos, las variables derivadas seleccionadas se calcularon solamente a partir de los datos de área o de diámetro (estimado a partir del área, suponiendo un conducto de sección circular).

Relativas a todos los vasos medidos se seleccionaron el área total (TVA), el número de vasos (NV) y la conductividad específica de la madera temprana (KS); ésta última obtenida de acuerdo con la ley de Hagen-Poiseuille (Tyree & Zimmermann 2002), atendiendo a la expresión matemática $KS = (\pi \varrho / 128 \eta A) \sum_1^n d_t^4$, donde ϱ es la densidad del agua a 20 ºC (998.2 kg m^{-3}), η es la viscosidad del agua a 20 ºC (1.002 x 10^{-9} MPa), A es el área de madera temprana analizada en el año t, y d_t es el diámetro de cada uno de los n vasos medidos en el año t. TVA y NV fueron estandarizadas a un marco de 10 mm de la anchura tangencial, mientras que el área de madera temprana para calcular KS fue estimada como el polígono convexo que envuelve a todos los vasos. De las variables de área media (MVA), área media de los vasos mayores del percentil 90 % de cada anillo (M90) y diámetro ponderado hidráulicamente (HYD), fueron elegidas tanto las relativas al total de los vasos medidos como a la primera hilera (*r1*) o al resto de vasos de madera temprana (*nr1*), ya que en ocasiones la selección de los vasos más grandes del anillo, que son los formados al inicio de la estación, mejora notablemente la señal climática (García-González & Fonti 2006; González-González et al. 2014). El diámetro hidráulicamente ponderado, ó diámetro hidráulico (HYD) fue calculado como $\sum_1^n d_t^5 / \sum_1^n d_t^4$, para n vasos de diámetro d en el año t, siendo proporcional a la conductividad hidráulica del xilema (Sperry et al. 1994; Fichot et al. 2009).

Variable de crecimiento	*Abreviatura*	*Unidades*
Anchura total del anillo	**RW**	µm
Anchura de la madera temprana	**EW**	µm
Anchura de la madera tardía	**LW**	µm
Número total de vasos o poros	**NV**	vasos/cm
Conductividad específica	**KS**	kg m^{-1} s^{-1} MPa^{-1}
Área total del lumen de los vasos (>10.000 µm^2)	**TVA**	µm^2
Área media del lumen de los vasos (>10.000 µm^2)	**MVA**	µm^2
Área media del lumen de los vasos mayores del 90% del anillo	**M90**	µm^2
Diámetro ponderado hidráulicamente (>10.000 µm^2)	**HYD**	µm
Área media del lumen de los vasos de la primera hilera	**MVA-r1**	µm^2
Área media del lumen de los vasos mayores del 90% de la primera hilera	**M90-r1**	µm^2
Diámetro ponderado hidráulicamente de los vasos de la primera hilera	**HYD-r1**	µm
Área media del lumen de los vasos no pertenecientes a la primera hilera	**MVA-nr1**	µm^2
Área media del lumen de los vasos mayores del 90% de los no pertenencientes a la primera hilera	**M90-nr1**	µm^2
Diámetro ponderado hidráulicamente de los vasos no pertenecientes a la primera hilera	**HYD-nr1**	µm

Tabla 5. Variables de crecimiento consideradas en la Tesis, tanto anatómicas como de anchuras

3.3.3 Análisis de datos

3.3.3.1 Caracterización de las series individuales

Las series individuales, antes y después del proceso de estandarización, fueron caracterizadas mediante una serie de parámetros estadísticos frecuentemente empleados en dendrocronología (Fritts 1976). Estos estadísticos aportan información preliminar sobre la aptitud de las series para análisis dendroclimatológicos y orientan sobre el método de estandarización a utilizar.

De esta manera, se emplearon:

- **Media aritmética (*Avg*)**: Representa el valor medio de la variable a estudiar para todos los anillos de una misma muestra.

- **Desviación típica (*Std*)**: Expresa la dispersión que experimentan los valores medidos en los anillos con respecto a la media.

- **Coeficiente de variación (*CV*)**: Cociente entre la desviación típica y la media. Es una medida de la variabilidad dentro de las series.

• **Coeficiente de sensibilidad media (*MS*)**: Es un estadístico muy utilizado en análisis dendrocronológicos (Douglas 1935), que mide la variación entre un anillo de crecimiento y el siguiente, indicando la variabilidad interanual, sobre todo de alta frecuencia (Fritts 1976; Xiong & Palmer 1995). Los valores bajos reflejan una escasa variación interanual en la variable de estudio, lo que conlleva una difícil sincronización (serie complaciente), mientras los valores altos (serie sensible) corresponden a una importante variación interanual. Habitualmente oscila entre 0,2 y 0,3, con un valor teórico entre 0 y 2 (Fritts 1976).

• **Coeficiente de autocorrelación de primer orden (*AR1*)**: Indica la correlación que existe entre cada valor de la serie y el que le precede, por tanto mide la influencia del crecimiento previo. Cuando el crecimiento de un año está fuertemente influido por el del año anterior, las tendencias se prolongan durante largos períodos de tiempo, razón por la cual este coeficiente es una medida de las variaciones de baja frecuencia.

3.3.3.2 Eliminación de tendencias individuales: estandarización

Antes de proceder al cómputo de la cronología media poblacional para cada variable, fue necesario transformar los datos brutos de cada individuo para que fuesen comparables entre sí, mediante el proceso de **estandarización** (Cook et al. 1992). Esto es debido a que la información que recogen los anillos es resultante de una combinación de influencias endógenas (fisiológicas), exógenas (climáticas, dinámica forestal, perturbaciones, etc.) y restricciones geométricas impuestas por el crecimiento, algunas de las cuales deben ser minimizadas para permitir posteriores análisis. Mediante la estandarización se persigue minimizar las tendencias no deseadas presentes en las series (Cook et al. 1992), obteniendo unos valores finales de media 1 para cada árbol, conocidos como índices de crecimiento (Fritts 1976), y que son posteriormente promediados en la cronología final de cada población. Generalmente, estas tendencias son debidas a la edad y a la dinámica forestal, e inherentes a cada individuo.

En todos los artículos de esta Tesis se empleó el mismo método de estandarización, así como para cada una de las variables estudiadas, tanto para las anchuras como para las variables anatómicas derivadas de los elementos conductores. Se ajustó un *spline* cúbico de suavizado a cada una de las series individuales, ya que ésta es un función flexible que se adapta a una gran diversidad de tendencias generales de las variables estudiadas. De esta manera fue posible estandarizar de forma eficaz tanto las variables anatómicas, cuya tendencia era ascendente especialmente en la madera juvenil, como las anchuras, que se aproximaban sobre todo a la tendencia seguida por una función exponencial negativa. Los índices de crecimiento fueron obtenidos mediante el cociente entre el valor para cada año y el estimado por la función. Para todas las series la función *spline* elegida fue bastante flexible, reduciendo en un 50% un ciclo de 32 años, ya que no era necesario mantener ciclos de baja frecuencia para posteriores análisis.

El ajuste de las funciones *spline* para la estandarización se realizó mediante rutinas de programación elaboradas con EMBARCADERO DELPHI XE7, empleando los estadísticos indicados por Cook et al. (1992) en los capítulos 3.3 y 3.4. Finalmente, no se aplicó ningún tipo de modelado autorregresivo sobre los índices resultantes, ya que en el caso de las variables anatómicas no existía autocorrelación estadísticamente significativa.

3.3.3.3 Cómputo y caracterización de la calidad estadística de la cronología

Una vez realizada la estandarización, los índices de crecimiento de cada individuo fueron promediados mediante una media aritmética, obteniendo así una cronología media para cada variable de crecimiento o anatómica en cada una de las localidades estudiadas. A continuación se caracterizó la calidad de las cronologías mediante el cómputo de determinados estadísticos al uso en

dendrocronología. Esta caracterización se realizó para un período de tiempo cubierto por al menos ocho-diez árboles en cada localidad, que es lo que se conoce como *intervalo común*. Los estadísticos calculados fueron:

- **Correlación media entre árboles (*Rbt*)**: Es el valor empleado con más frecuencia para medir la señal de una cronología, y habitualmente se conoce como señal común. Se trata del promedio de las correlaciones entre las series de índices de todos los individuos para el intervalo común.

- **Varianza explicada por el primer autovector (*%Var*):** Es otra medida de la señal común, independiente del número de árboles, y se obtiene a partir de un análisis de componentes principales aplicado al conjunto de las series individuales.

- **Relación señal-ruido (*SNR*)** (Graybill 1982): Es el resultado de dividir la varianza explicada por el primer autovector por la medida estadística que constituye el ruido. Es muy dependiente del número de árboles empleado, ya que el ruido decrece a medida que se aumenta el número de series. También fue utilizada para elegir la función *spline* en estandarización (Briffa 1984).

- **Concordancia de la cronología con la población (*EPS*)**: Es un parámetro calculado a partir de la correlación media entre árboles que compara la señal obtenida con la de una población teórica formada por un número infinito de muestras. Indica en qué porcentaje la cronología obtenida es representativa de la población, expresada normalmente en tanto por uno. Al igual que la relación señal-ruido, es muy sensible al número de árboles que incluye la cronología.

3.3.3.4 Comparación entre cronologías

Las cronologías de cada variable fueron comparadas entre sí, tanto estadística como visualmente, tratando de determinar el grado de similitud en la señal climática que presentaban. Estas comparaciones se hicieron en base al grado de sincronización entre cronologías, basándose en el uso del **coeficiente de correlación de Pearson (*r*),** un estadístico paramétrico que puede ser utilizado para medir el grado de asociación entre dos series temporales. Se emplea asimismo su nivel de significación, el cual es obtenido mediante el cómputo de la *t* de Student a partir del coeficiente de correlación, cuyo uso está muy extendido en dendrocronología (Baillie & Pilcher 1973).

3.3.3.5 Relaciones clima-crecimiento

Para el establecimiento de las relaciones clima-crecimiento se utilizaron funciones de correlación, definidas como el cálculo de todas las correlaciones entre las cronologías y los registros meteorológicos a lo largo de la estación de crecimiento (Blasing et al. 1984). Estas funciones se calcularon utilizando el coeficiente de correlación de Pearson (*r*), aunque su significación fue asignada mediante 10.000 iteraciones de la técnica bootstrap (Guiot 1991) para cada coeficiente, y aplicando la corrección propuesta por Mason and Mimmack (1992) para los intervalos de confianza. El motivo de emplear esta corrección se debe a que con frecuencia el coeficiente de correlación obtenido por bootstrap es un estimador sesgado del coeficiente de correlación, y presenta intervalos de confianza asimétricos.

El cálculo de estas funciones de correlación se llevó a cabo para diferentes períodos en función del capítulo del que se tratara, pero en todos los casos se pudo disponer de un registro completo de datos climáticos.

Los datos climáticos fueron obtenidos del *KMNI Climate Explorer* (http://climexp.knmi.nl/). Se trata de una aplicación para la investigación climática, administrada por el Instituto Meteorológico de Holanda (KNMI) y que contiene una amplia colección de conjuntos de datos climáticos y herramientas de análisis de alta resolución. De toda esta colección de datos, se utilizaron mayoritariamente los

procedentes de las bases de datos del *Climate Research Unit* (CRU) de la Universidad de East Anglia (http://www.cru.uea.ac.uk/). Es una serie de conjuntos de datos globales sobre estaciones meteorológicas que el CRU ha compilado y actualizado durante los últimos 30 años. A partir de esta información, (New et al. 1999) construyeron una cuadrícula o *grid* climatológica de 0,5º × 0,5º de resolución (CRU CL 1.05), de medias mensuales de determinadas variables para el período 1961–1990. Una *grid* climatológica representa el clima promedio para un periodo de tiempo largo y permite la comparación espacial de características ambientales relacionadas con el clima, pero no recoge la variación temporal. Esta *grid* fue ampliada y revisada por diferentes autores (Mitchell et al. 2004; Mitchell & Jones 2005) hasta llegar a la más actualizada (CRU TS 3.22), elaborada por Harris et al. (2014) para el período 1901-2013, que es la que se utilizó para obtener la práctica totalidad de las variables climáticas con las que se ha trabajado en esta Tesis. Se obtuvieron, para cuadrantes territoriales de 0,5º × 0,5º de resolución, las siguientes variables climáticas:

1. **P:** precipitación (mm, equivalente a litros/m2)
2. **T:** temperatura media del aire (Cº)
3. **Tmin:** temperatura mínima del aire (Cº)
4. **Tmax:** temperatura máxima del aire (Cº)
5. **Tamp:** Amplitud térmica (Cº)
6. **Vap:** presión de vapor (hPa)
7. **PDSI:** Índice de sequía de Palmer

También se utilizó la presión atmosférica (**SLP**, en mb), que se obtuvo de otra base de datos disponible en el *KMNI Climate Explorer*, la *Monthly Northern Hemisphere sea level pressure data set*, del *CISL Data Archive*. Esta variable también se obtuvo para cuadrantes de 0,5º × 0,5º de resolución, cubriendo el período 1899-2013.

Los meses empleados para el análisis fueron aquellos que *a priori* se consideró que podían tener cierta influencia sobre el crecimiento. En el caso de las variables de madera temprana fue seleccionado un período total de 13 meses; desde Mayo del año anterior a la formación del anillo hasta Mayo del año de crecimiento, mientras que para la anchura total y la madera tardía el período empleado se extendió a 18 meses, hasta Octubre del año en curso. Además de analizar las respuestas al clima para los meses individuales, se tuvieron en cuenta una serie de agrupaciones mensuales con el objetivo de obtener valores estacionales, cuando alguna de las variables climáticas estudiadas (en especial la temperatura media de un período o la precipitación acumulada) pudieran tener mayor influencia sobre el crecimiento que los valores mensuales.

3.3.4 Análisis de datos específicos para determinados Artículos

3.3.4.1 Estimación de la edad cambial

En el Artículo V de Resultados se evaluó la tendencia del tamaño de vasos con la edad, utilizando para ello una cronología de 500 años. Para ello se compararon los árboles en base a la edad cambial y no a la edad de datación, siendo necesario estimar las edades reales de aquellos cuyos *cores* no incluían la médula debido a las frecuentes roturas y oquedades o por las grandes dimensiones de muchos de ellos, que impidieron la extracción de *cores* completos. En estos casos se siguieron dos métodos de estimación diferentes según el aspecto que presentaran los *cores*. Para aquellos en los que se pasó cerca de la médula y en los que se podían visualizar los arcos de los anillos interiores, la estimación

de la posición del centro cronológico y de los anillos perdidos se llevó a cabo siguiendo el método gráfico-matemático combinado propuesto por Rozas (2003). Si los arcos no estaban presentes, la longitud perdida fue calculada mediante la diferencia entre el radio geométrico del fuste y la longitud total del *core*, estimando el número de anillos perdidos como la extrapolación de la tasa de crecimiento radial medio de los veinte últimos anillos del *core*.

3.3.4.2 Identificación de perturbaciones antrópicas

En el Artículo IV de Resultados se llevó a cabo un estudio de las variaciones en el crecimiento radial observadas en algunas de las localidades, con el objetivo de identificar posibles perturbaciones, ya que todas ellas evidenciaron manejo reciente. Para ello se analizó la frecuencia de cambios bruscos en el crecimiento, conocidos como liberaciones y reducciones, sobre las series de anchura total, utilizando el método del Porcentaje de Cambio de Crecimiento (PGC) propuesto por Nowacki and Abrams (1997) y adaptado para el estudio de los robles desmochados en el Norte de España por Rozas (2005a). La aplicación de esta metodología permite conocer la proporción de árboles que muestran variaciones bruscas de crecimiento por década, aportando así valiosa información acerca de la dinámica de las masas consideradas.

3.3.4.3 Comparación entre cronologías

En los Artículos III, IV y V de Resultados, además del coeficiente de correlación de Pearson (*r*), debido al gran número de variables a comparar se utilizó también el **análisis de componentes principales (ACP)** con el fin de determinar las diferencias en la señal aportada por cada una de ellas. Se trata de una técnica ampliamente utilizada en estudios ecológicos, que permite tanto reducir el número de variables como identificar la similitud entre las mismas. En estos casos, se partió de una matriz constituida por el conjunto de las series de índices de crecimiento anuales (variables) y el conjunto de años cubiertos por la totalidad de las series (casos), obteniéndose así la matriz de correlaciones entre todas las variables. Mediante combinación lineal de las variables originales, se obtuvieron los autovectores o componentes principales, ortogonales entre sí. La ordenación de las variables originales en el espacio definido por los autovectores permitió establecer el grado de analogía entre ellas, y así determinar cuáles aportaban información redundante, y cuáles información exclusiva. El análisis de componentes principales se aplicó sobre todas las variables anatómicas, así como para las anchuras de la madera temprana, tardía y total del anillo.

En los Artículos I y V, donde se analizaron dos poblaciones muy cercanas y dos tipologías de árboles de la misma población respectivamente, el **ACP** se aplicó también sobre la matriz de correlaciones de todos los árboles muestreados, tratando así de analizar la ordenación de los árboles individuales para las variables estudiadas en cada uno de los casos, visualizando las diferencias o similitudes entre grupos.

En los Artículos III y IV, en los que se estudiaron un conjunto de localidades que integran un gradiente en el primero y un transecto en el segundo, el **ACP** fue utilizado también para estudiar las relaciones entre dichas poblaciones y para cada variable seleccionada, aplicado sobre la matriz de correlaciones de cada grupo de localidades.

3.3.4.4 Análisis temporal de la señal común

En el Artículo V de Resultados, se estudiaron las fluctuaciones de la calidad estadística de las cronologías a lo largo del tiempo a través del análisis de la variación de los estadísticos del intevalo común durante el período 1626-2003. Para ello se períodos de 50 años desplazados un año en cada paso.

3.3.4.5 Relaciones clima-crecimiento

En los Artículos I y II de Resultados, las funciones de correlación utilizadas para establecer las relaciones clima-crecimiento también fueron calculadas para los árboles individuales, ya que se ha demostrado que el estudio de las respuestas individualizadas en ocasiones pueden aportar valiosa información adicional (Rozas & Olano 2013; Rozas 2015).

En cuanto a los datos climáticos utilizados para obtener las respuestas de cada localidad, en el Artículo I sólo los de temperatura procedieron del CRU. Para obtener los de precipitación se utilizaron series regionales extraidas de la estación meteorológica de Puebla de Sanabria, con un registro completo para 1945-2002. En este caso, las series estimadas de precipitación del CRU ofrecieron menor precisión que los datos locales, mientras que los de temperatura mostraron mayor fiabilidad. En el Artículo II, a las variables climáticas habituales se añadió el Índice de Precipitación-Evapotranspiración Estandarizado (SPEI) (Vicente-Serrano et al. 2010) para evaluar la influencia de la sequía estival en el crecimiento. Los valores de este índice fueron calculados en R (R Development Core Team 2013), usando el paquete 'SPEI' y considerando escalas temporales de 1 a 21 meses. Los efectos del SPEI se estudiaron a lo largo de un período de 20 meses, desde Mayo del año anterior hasta Diciembre del año en que se forma el anillo.

En el Artículo V, adicionalmente se calcularon funciones de correlación móviles (Biondi & Waikul 2004) utilizando períodos de 45 años desplazados un año, con el objetivo de estudiar la variación de la señal climática con la edad.

Tanto en éste como en el Artículo IV, a las variables climáticas analizadas habitualmente se añadió un parámetro macroclimático, la Oscilación del Atlántico Norte (NAO). Es una fluctuación a gran escala en la masa atmosférica situada entre la zona de altas presiones subtropicales y la baja polar en la cuenca del Atlántico Norte, cuya influencia se extiende desde Norteamérica central hasta Europa y gran parte del Norte de Asia, determinando la variabilidad del clima invernal en la región del Atlántico Norte. Para este parámetro se obtuvieron índices mensuales de la base de datos del CRU (http://www.cru.uea.ac.uk/cru/data/nao/) durante el período 1821-2015, basados en el observatorio de Gibraltar.

3.3.4.6 Filtrado de los datos

De acuerdo con (García-González and Fonti (2006)), no todos los vasos de primavera son indicadores de la misma señal ecológica, ya que con frecuencia los vasos de diferente tamaño se formaron en distinto momento a lo largo del período de crecimiento, y por tanto pueden haber sido influenciados por diferentes condiciones ambientales. En general, los vasos de mayor tamaño son los que registran la señal climática de mayor calidad, de modo que la eliminación progresiva de aquellos de menor tamaño, atendiendo a diferentes criterios, puede mejorar sensiblemente la relación entre el tamaño de vasos y una determinada variable meteorológica.

El análisis por separado de distintas filas responde parcialmente a este objetivo, pero siempre incluye vasos pequeños y no distingue entre vasos diferenciados en distintos momentos. Por este motivo, en el Artículo I de Resultados se llevó a cabo un filtrado progresivo de los vasos de cada anillo por percentiles. El filtro fue aplicado en dos direcciones: por un lado se eliminaron gradualmente los vasos más pequeños, reteniendo la señal contenida en los más grandes, mientras que por el otro se llevó a cabo el procedimiento opuesto, eliminando los vasos más grandes. Estos cálculos se basaron en la distribución del tamaño de vasos dentro de cada anillo, suprimiendo en cada paso el 1% de los más grandes o más pequeños, respectivamente. Después de la eliminación de ese percentil, se recalcularon las series individuales de la variable estudiada con el *n*-porcentaje de vasos que fueron retenidos, así como las correspondientes cronologías medias y los consiguientes análisis de las relaciones con el clima.

Para llevar a cabo este gran volumen de cálculos se desarrolló una rutina de programación específica usando Embarcadero Delphi XE7. Este método aportó información crucial para abordar la interpretación de las relaciones clima-crecimiento.

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Resultados

4

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4 RESULTADOS

4.1 SÍNTESIS DE LOS RESULTADOS OBTENIDOS

Los resultados de esta Tesis Doctoral se presentan estructurados en cinco artículos, que se explican de forma resumida a continuación.

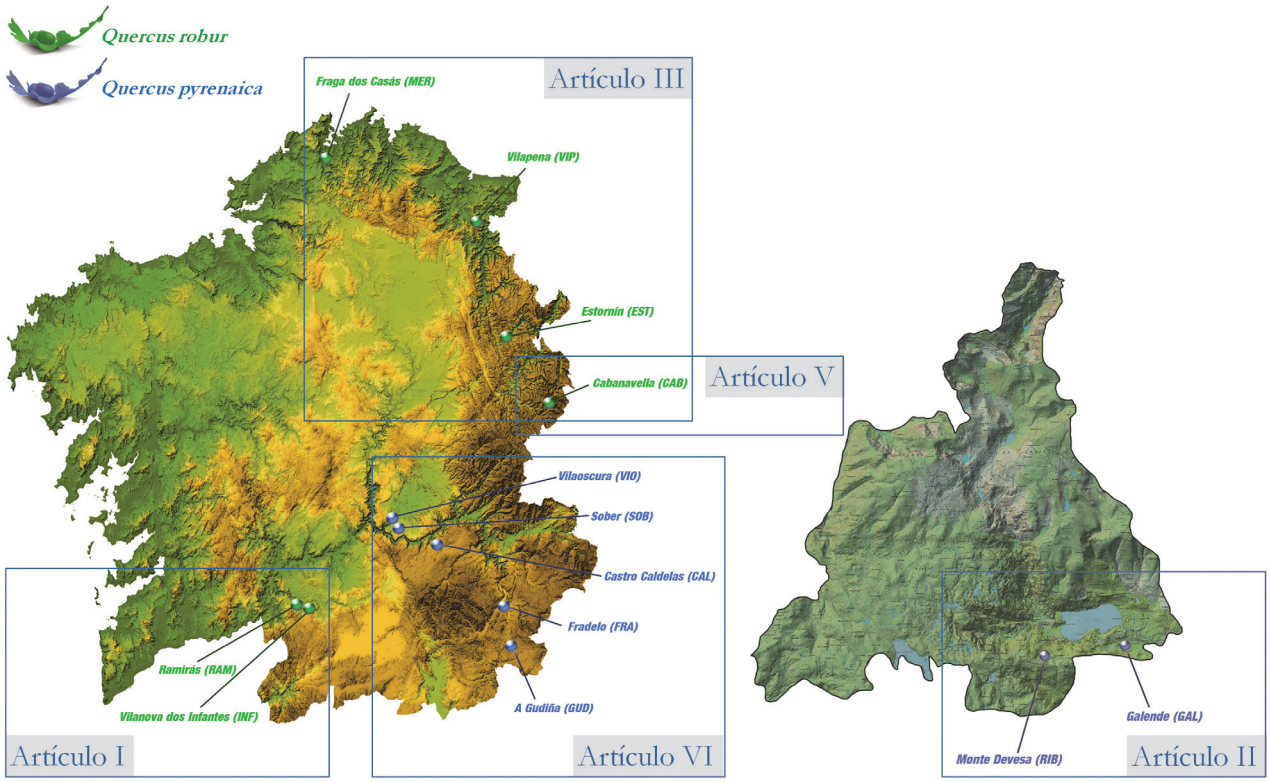

Figura 1. Localidades que integran cada uno de los capítulos de Resultados de la Tesis Doctoral

Artículo I. El estrés de invierno controla el crecimiento radial y la anatomía de la madera temprana de *Quercus robur* L. en su límite sur de distribución en el Noroeste de la Península Ibérica

En este Artículo se estudiaron los anillos de crecimiento en dos localidades próximas de *Quercus robur* en su límite sur de distribución (Figura 1), con una fuerte influencia mediterránea durante los meses de verano, pero con un régimen de precipitación atlántico en invierno. Ambas están sometidas a distinto grado de humedad edáfica, ya que Vilanova dos Infantes (INF) se encuentra en una ladera de pendiente moderada, mientras que Ramirás (RAM) se ubica en una pequeña vaguada por la que discurre un riachuelo. El crecimiento radial se caracterizó mediante series dendrocronológicas de vasos de la madera temprana, así como el incremento de la madera tardía (LW). Los vasos primaverales se estudiaron a través de su área media, dividiéndola entre aquella correspondiente a los vasos de la primera fila (MVA-r1) y la del resto de vasos (MVA-nr1). Estas series se elaboraron a partir de 11 árboles de cada localidad y los análisis fueron llevados a cabo para el período 1956-2013.

Los resultados obtenidos mostraron pequeñas diferencias de tamaño y distribución de los vasos de cada localidad, destacando la mayor heterogeneidad observada en los vasos de la primera fila en INF. Las relaciones de los vasos de madera temprana con series climáticas de precipitación y temperatura mostraron un claro efecto negativo de las condiciones de invierno sobre el crecimiento en las dos

localidades. Ambas registraron fuertes correlaciones con la temperatura durante la dormición, es especial durante los meses de diciembre y enero; sin embargo, la influencia de la precipitación fue especialmente acusada en RAM, la localidad más húmeda. Estas respuestas fueron recogidas por los vasos de la primera fila en ambas, pero en RAM la influencia de la señal se prolongó en el resto de vasos primaverales; en INF, por el contrario, el efecto del invierno prácticamente no se apreció en estos vasos, sino que se revelaron más sensibles a las condiciones de mayo, respondiendo positivamente a la temperatura y negativamente a la precipitación, aparentemente en el momento de su formación. Las respuestas de LW fueron mucho más débiles y relacionadas con la estación de crecimiento previa, aunque en INF se encontró cierta relación con la precipitación durante septiembre del año de formación del anillo, indicando un pequeño pulso de crecimiento al final de la estación si las condiciones son favorables.

Con el fin de entender mejor las respuestas encontradas y las diferencias entre localidades en relación con el régimen hídrico del suelo, se utilizó el Índice Estandarizado de Precipitación-Evapotranspiración (SPEI) para estudiar la respuesta de los árboles en varias escalas temporales. En el presente estudio, este índice registró los efectos del exceso hídrico en cada localidad y cada variable estudiada de manera mucho más precisa, aportando también información acerca del alcance de los mismos. En RAM la influencia negativa de la saturación hídrica invernal del suelo sobre los vasos más grandes (primera fila) se prolongó durante toda la estación, afectando los eventos tanto de corta como de larga duración, pero sobre todo a una escala temporal de seis meses. Por el contrario, el exceso hídrico en INF afectó únicamente al crecimiento de los vasos que no estaban en la primera fila, siendo necesarios períodos húmedos de más larga duración tanto en invierno como durante la estación de crecimiento para que se manifestaran sus efectos. El crecimiento radial en ambas localidades estuvo favorecido por los períodos húmedos de larga duración, pero con mayor intensidad en INF, acusando el efecto durante todo el crecimiento activo.

Los resultados del trabajo parecen indicar que la formación de madera en estos robles, situados en el límite con la región mediterránea, está claramente controlada por el balance de carbohidratos durante la estación de crecimiento. La anatomía de la madera temprana, que determina las principales relaciones hídricas de los árboles, refleja el consumo de carbohidratos para mantener la respiración durante la estación de dormición. Sin embargo, este consumo de carbohidratos es mucho mayor en la localidad sometida a encharcamiento, ya que las condiciones de hipoxia que se pueden producir durante el invierno acarrean un consumo de reservas mucho mayor. Por otra parte, los árboles parecen adoptar una estrategia conservadora para evitar la sequía, ya que durante los meses más secos parecen priorizar la reserva de carbohidratos para la estación siguiente en detrimento del crecimiento radial durante el verano. Por tanto, la fuerte respuesta a las condiciones del invierno parece estar ligada al hecho de que la asimilación durante la estación de crecimiento es limitada debido a la fuerte tendencia mediterránea durante los meses de verano.

Artículo II. El área de los vasos de *Quercus pyrenaica* Willd. es un potente indicador del exceso de agua en el suelo al inicio del crecimiento

En este Artículo se estudió el potencial de los vasos primaverales como indicadores, evaluando su sensibilidad para registrar condiciones ecológicas locales. Para ello se utilizó como variable de crecimiento el área media de los vasos de la madera temprana (MVA), que también fueron comparados frente a la anchura total del anillo (RW). Se seleccionaron dos localidades de *Quercus pyrenaica* bajo condiciones de elevada humedad edáfica durante el invierno en las cercanías del Parque Natural del Lago de Sanabria y Alrededores (Figura 1), cerca del límite norte de distribución de la especie, que presentaban grandes diferencias en cuanto a la duración del período de saturación de agua durante el invierno. Monte Devesa (RIB) se encuentra situado en una ladera de moderada pendiente en la que predominan las condiciones de escorrentía sobre un suelo somero, mientras Vegas de Galende (GAL) se ubica por el contrario en un fondo de valle en el que las condiciones de encharcamiento son

frecuentes durante los meses de mayor pluviometría y favorecido por una capa freática a poca profundidad, ya que la mayor parte de la depresión presenta un suelo turboso. Para el análisis se elaboraron series dendrocronológicas a partir de 10 árboles en cada localidad, y se estudiaron a lo largo del período 1937-2002.

A pesar de que ambas localidades se encuentran muy cercanas entre sí, y que presentaron distribuciones y tamaños de vasos similares, la comparación de las series individuales de los árboles de cada localidad, realizada mediante una ordenación en componentes principales, reveló una clara discriminación entre ambas, tanto para MVA como para RW, apuntando una clara diferencia en la respuesta ambiental entre las dos poblaciones para las dos variables estudiadas. Pese a la mayor calidad estadística de las cronologías de RW, fue el estudio del tamaño de los vasos el que permitió establecer las relaciones existentes con el clima y las diferencias entre ambos sitios. La existencia de reducciones sincrónicas de crecimiento para RW, probablemente debidas a acontecimientos no climáticos, impidió que esta variable presentase una respuesta al clima; sin embargo, estas reducciones de crecimiento no afectaron a las cronologías de MVA.

La principal respuesta encontrada fue el efecto negativo de la precipitación en primavera, pero que fue registrada en distintos momentos para cada una de las localidades, debido a las diferencias topográficas existentes. La mayoría de los árboles de RIB recogieron intensamente esta señal en abril, junto con una respuesta menos fuerte de signo positivo a la temperatura, mientras que en GAL la respuesta tuvo lugar en mayo, un mes más tarde, siendo expresada por menos de la mitad los árboles.

El análisis de las relaciones clima-crecimiento a través de subconjuntos de vasos permitió comprender mejor las respuestas obtenidas. Al separar los vasos por filas se vio que el efecto de la precipitación en RIB afectaba fundamentalmente a los vasos más grandes, es decir, los que se forman al principio de la estación de crecimiento, mientras que en GAL la señal climática se perdía por completo. Por ello, fue necesario llevar a cabo un filtrado progresivo de los vasos con el objetivo de localizar aquellos que maximizaran la respuesta en esta localidad, eliminando en cada paso un porcentaje de los vasos más grandes o más pequeños, según la dirección del filtro, y recalculando las relaciones clima-crecimiento en cada paso. Los análisis confirmaron que sólo los vasos mayores, formados al inicio de la estación, recogían la señal en RIB, mientras que en GAL la respuesta estaba más concentrada en vasos intermedios.

Los resultados de este trabajo sugieren que en el efecto del encharcamiento retarda o ralentiza el inicio de la estación de crecimiento en la localidad más húmeda; igualmente, también produce una menor variación intra e interanual, que se muestra en una menor señal común y respuesta al clima en GAL. Por lo tanto, se confirma que los vasos primaverales presentan una gran sensibilidad como indicadores ecológicos, pudiendo registrar tanto señales regionales como locales; pero también indican que para aprovechar esta sensibilidad, es necesario llevar a cabo un minucioso análisis, con métodos que permitan optimizar las señales obtenidas.

Artículo III. Análisis dendrocronológico de las adaptaciones de los vasos primaverales de *Quercus robur* L. a lo largo de un gradiente altitudinal en el noroeste de la Península Ibérica

En este Artículo se ha estudiado el potencial de las variables de crecimiento para estudiar las relaciones con el clima y las posibles adaptaciones anatómicas de *Quercus robur* a lo largo de un gradiente altitudinal creciente desde la costa Norte hasta las montañas orientales de Galicia (Figura 1). Así, se hizo una comparativa de las localidades de Fraga dos Casás (MER), Vilapena (VIP), Estornín (EST) y Cabanavella (CAB), todas bajo un clima atlántico con elevada pluviosidad, concentrada sobre todo en otoño e invierno y sin apenas sequía estival, pero con diferencias en cuanto a temperaturas medias anuales (más altas en las localidades de baja altitud) y oscilación térmica/continentalidad (más acusadas

cuanto más lejos de la costa), así como relativas a la longitud del período vegetativo. En estas localidades se llevó a cabo un seguimiento fenológico con el fin de interpretar correctamente las respuestas climáticas. Los análisis fueron realizados para el período 1954-2003, cubierto por al menos ocho árboles de cada una de las localidades.

Los tamaños de vasos fueron mayores en los límites del gradiente (MER y CAB), apreciándose una tendencia ascendente del tamaño con la altitud excepto para MER. Los vasos pequeños, predominantes en todas las localidades, fueron más abundantes en las situadas a media altitud (VIP, EST), incluso en la primera fila. Para cada localidad se llevó a cabo un análisis de componentes principales (ACP) sobre las 15 variables de crecimiento calculadas inicialmente, identificando tres grupos distintos relacionados con diferencias en la información ecológica aportada, pero con muchas variaciones de ordenación en función del sitio estudiado. En vista de estos resultados, se seleccionó LW para caracterizar el crecimiento del anillo, y tres parejas de variables anatómicas poco relacionadas entre sí (NV y TVA vs. KS y MVA vs. HYD-r1 e HYD-nr1) pero con ciertas similitudes dentro de cada grupo, para llevar a cabo los análisis posteriores y el estudio de las diferentes relaciones con el clima. La caracterización estadística mostró valores bajos de señal común para todas ellas con la excepción de LW.

Los resultados de la ordenación de las localidades mediante ACP para todas las variables mostraron un comportamiento diferente de MER excepto para HYD-nr1, mientras que el resto de sitios tendieron a ordenarse según el gradiente altitudinal, con algunas diferencias según la variable. Las diferencias observadas en MER parecen ser debidas tanto a la existencia de un clima más cálido y húmedo, como a posibles diferencias en las condiciones del sitio. El estudio de las relaciones clima-crecimiento se llevó a cabo comparando las respuestas recogidas por cada pareja de variables anatómicas, además de las registradas por LW. Pese a la gran correlación existente entre NV y TVA, o entre MVA y KS, no expresaron relaciones similares con el clima en la mayoría de los casos y éstas fueron, en general, débiles y poco significativas para ambos grupos, relacionadas con las condiciones de primavera y de la estación de crecimiento previa. Destacó en intenso efecto de la precipitación de Mayo en MER, registrada por NV y KS, y más débilmente por MVA, y las respuestas opuestas de KS a las temperaturas máximas de Abril en MER y CAB. Pese a todo, no se adivinó ninguna tendencia clara debida al gradiente altitudinal. Los mejores resultados fueron los relativos a HYD-r1 donde se pudo ver claramente el comportamiento diferenciado de MER, que registró un efecto negativo de las temperaturas de la dormición frente a la relación positiva con las temperaturas de la primavera del resto de localidades. Las respuestas de LW tampoco mostraron ningún patrón espacial y estuvieron sólo ligeramente ligadas a la temperatura excepto para MER, relacionadas además con las precipitaciones de verano. Recogió también cierta influencia negativa de las temperaturas máximas estivales y una respuesta positiva a las temperaturas máximas de primavera, efecto también observado en VIP y EST pero un mes más tarde y con menor intensidad; EST y CAB por otro lado mostraron correlaciones positivas con las temperaturas del final de la estación (Septiembre).

Este trabajo pone de manifiesto la dificultad para el estudio de las relaciones clima-crecimiento en un área oceánica cuando no existe un único factor limitante del crecimiento en la mayoría de los años.

Artículo IV. Los vasos primaverales y la anchura de madera tardía explican el papel del clima sobre la formación de la madera de *Quercus pyrenaica* Willd. a lo largo del límite atlántico-mediterráneo en el noroeste de la Península Ibérica

En este Artículo se estudió la variabilidad de respuesta de la anatomía de cinco localidades de *Quercus pyrenaica* sometidas a diferentes condiciones ambientales y bajo distintos grados de perturbación, que conforman un transecto altitudinal Noroeste-Este en Galicia (Figura 1). Así se muestrearon, de menor a mayor altitud y con diferentes regímenes térmicos y pluviométricos, las localidades de

Villaoscura (VIO) y Sober (SOB), ambas en los límites de una depresión terciaria con marcado carácter mediterráneo y acusada sequía estival, Castro Caldelas (CAL), a media altitud y en una posición topográfica de ladera donde el período de sequía estival es más corto, Fradelo (FRA), situada en una ladera cercana a un embalse donde son frecuentes las nieblas y la sequía de verano es algo más acusada que en la anterior localidad, y A Gudiña (GUD), que es la localidad más elevada, situada en una ladera donde las lluvias de invierno son abundantes y la sequía estival, aunque presente, es de menor duración e intensidad. En estas localidades se llevó a cabo un seguimiento fenológico para integrar la información recogida con la interpretación de las respuestas climáticas. Todos los análisis se realizaron para los períodos 1953-2012 y 1967-2012 (VIO), este último debido a la corta longitud de las series de la localidad.

Previamente al resto de análisis se realizó un estudio de las relaciones existentes entre 15 variables calculadas a partir de los vasos y las anchuras del anillo, mediante un análisis de componentes principales. De acuerdo con los resultados de dicho análisis, se seleccionaron para este trabajo el diámetro hidráulico de los vasos de la primera fila (HYD-r1) y del resto de vasos primaverales (HYD-nr1) para caracterizar la madera temprana, y se añadió el área media de vasos (MVA) para comparar los resultados frente a trabajos anteriores que empleaban esta variable. La anchura de madera tardía (LW) se incluyó para describir el incremento de crecimiento radial.

Los resultados mostraron ciertas diferencias en las distribuciones y vasos de cada localidad. Se observó mayor cantidad de vasos en GUD y menor en VIO, siendo muy similar en el resto de localidades; VIO sin embargo registró mayor tamaño de vasos en la primera fila, contrastando con el menor tamaño en CAL. Por otro lado, la abundancia de vasos pequeños decreció progresivamente de VIO a GUD. La calidad estadística de las cronologías fue baja, algo habitual para los vasos pero inusual para las anchuras, de hecho aunque los mejores valores de señal común fueron los de LW, únicamente alcanzó un valor destacable en FRA. Esta falta de señal común fue asociada con la presencia de numerosas variaciones bruscas del crecimiento, identificadas como perturbaciones de origen antrópico en las localidades más bajas y relacionadas con incendios recurrentes en GUD, siendo FRA la menos afectada. Las cronologías de LW presentaron en conjunto una buena respuesta al clima, siendo la disponibilidad hídrica durante el final de la primavera y el inicio del verano la principal variable que controla su formación; no obstante, los resultados estuvieron parcialmente enmascarados por la dinámica observada en las masas, es especial VIO y CAL. Sin embargo, estas perturbaciones no afectaron a las cronologías de vasos, en las que se pudo apreciar (sobre todo en HYD) la importancia desempeñada por la distribución altitudinal de las localidades al ordenarlas según el análisis de componentes principales. Los resultados con el clima para el tamaño de vasos fueron muy pobres considerando MVA, por lo que fue necesario optimizar las respuestas analizando una variable más robusta (HYD) y separando los vasos según el momento de su formación; así se registraron notables diferencias entre localidades, sobre todo al utilizar la primera hilera (HYD-r1). Las localidades situadas a elevaciones medias recogieron intensamente el efecto negativo de las temperaturas mínimas al final de la dormición (en menor medida en VIO), efecto que se fue disipando con la altitud para dar paso a una señal positiva con las mínimas de principios de primavera, más intensa en la localidad más elevada (GUD). En FRA, aunque se registraron débilmente ambas respuestas, fue la precipitación del inicio de primavera la variable climática con mayor influencia en el tamaño de estos vasos, con un efecto beneficioso. Los vasos formados más tarde (HYD-nr1) mostraron una respuesta negativa con las temperaturas máximas de Mayo sólo en esta localidad, probablemente debido a que temperaturas elevadas aceleran la diferenciación y producen en consecuencia vasos de menor tamaño. En el resto de localidades estos vasos recogieron señales débiles, manifestando escasa relación con el clima. Se comprobó que las condiciones climáticas locales durante el invierno y gran parte de la primavera estaban influenciadas por las variaciones del índice NAO (Oscilación del Atlántico Norte), por lo que se calcularon las correlaciones con HYD para las diferentes filas de vasos. Se observaron respuestas negativas y altamente significativas, siguiendo un claro patrón altitudinal; las localidades más bajas registraron los efectos a finales del invierno y en los vasos de la primera fila, las localidades a media

altitud lo hicieron a principios de primavera, trasladando prácticamente toda la señal a los vasos formados más tarde en la estación, y GUD no registró ninguna respuesta ya que la NAO no parece ejercer efecto sobre el clima peninsular hacia finales de la primavera.

Los resultados de este trabajo ponen de manifiesto que *Q. pyrenaica* es una especie que presenta una clara respuesta al clima, la cual puede ser estudiada combinando las variables de madera temprana y tardía empleadas en este trabajo. Debido a su posición transicional hacia la región mediterránea se trata de una especie de gran importancia en el estudio del futuro comportamiento ante el cambio climático de los bosques ibéricos, así como para comprender los procesos que tienen lugar en estas áreas de transición.

Artículo V. Dependencia de la edad y tendencias temporales en una cronología de vasos primaverales de roble de 481 años de longitud

Con el objetivo de estudiar los posibles efectos de la edad en la anatomía de los robles, así como las tendencias a lo largo del tiempo, en este Artículo se analizaron dos grupos de edad diferentes dentro de la misma localidad. Se seleccionaron por un lado los árboles medianamente jóvenes (menos de 100 años) y por otro, los más viejos de la localidad de Cabanavella (CAB), una masa en la que predomina *Quercus robur* y que se encuentra próxima al límite altitudinal de distribución de la especie (Figura 1). Los efectos de la edad se evaluaron comparando ambos grupos en el período común 1938-2003, cubierto por al menos diez árboles de cada uno de ellos, mientras que las tendencias a lo largo del tiempo se analizaron a partir de la cronología correspondiente a los árboles viejos (1523-2003), para distintos períodos en función del análisis llevado a cabo.

Los resultados revelaron muy pocas diferencias en las características y distribuciones de vasos de ambas clases de edad, con la salvedad de que los árboles viejos presentaron mayor proporción de vasos en la primera fila, explicable por la mayor frecuencia de reducciones severas del crecimiento donde los anillos estaban formados por una única hilera de vasos. Al estudiar la tendencia del tamaño de vasos a lo largo de los casi 500 años que abarca la cronología de los árboles viejos, alineados según su edad cambial, se observó un predominio de vasos más pequeños en los árboles jóvenes, probablemente relacionado con la presencia de madera juvenil, y estabilizándose el tamaño a partir de los 20-30 años sin una tendencia clara a lo largo de todo el período. El estudio de las filas mostró el mismo patrón, aunque menos evidente en el caso de los vasos no pertenecientes a la primera hilera. Tampoco se apreciaron diferencias en las relaciones entre las 15 variables consideradas en este trabajo, analizadas según las lecturas hechas en los dos componentes principales, para los dos grupos de árboles estudiados. El análisis de estas relaciones a lo largo de un período más largo (1732-2003) mostró igualmente un patrón muy robusto y sin variaciones importantes, estableciéndose claramente tres grupos de variables bastante sólidos. En función de estas relaciones, analizadas en profundidad en los capítulos III y IV de Resultados, para los siguientes análisis se seleccionó como variable de crecimiento el diámetro hidráulico de los vasos de la madera temprana, pero diferenciando entre el correspondiente a los que integran la primera hilera (HYD-r1) y el del resto de vasos (HYD-nr1) con el objetivo de estudiar más en detalle el comportamiento de los distintos grupos de elementos conductores. Los resultados se compararon también con los de la madera tardía (LW).

La calidad estadística o señal común de estas variables no presentó diferencias significativas entre las clases de edad, pero sí que registró variaciones a lo largo del período 1626-2003, probablemente debido a los cambios ambientales de los últimos siglos, al hecho de la incorporación a la cronología de nuevos árboles en los períodos recientes, y también porque la tendencia con la edad no es la misma para todas las variables. La comparación de las cronologías de los dos grupos de árboles para cada una de estas variables evidenció de nuevo la gran similitud existente entre ambos, con altas correlaciones que indican escasa variación en la información recogida en cada caso. La ordenación de las series individuales de los árboles de cada clase de edad según los dos componentes principales reveló

que todos ellos se comportan como una única población, reflejado en las relaciones clima-crecimiento ya que ambos tipos registraron la misma respuesta positiva a las temperaturas de Abril en los vasos de la primera fila, mientras que las señales recogidas por el resto de vasos primaverales fueron poco significativas y débiles. También se identificó una clara influencia negativa de la NAO (Oscilación del Atlántico Norte) durante los meses de invierno (Diciembre-Enero) sobre todos los vasos primaverales, pero esta respuesta solo fue recogida por los árboles viejos. Las relaciones de LW con estas variables e índices climáticos fueron en todos los casos débiles y poco significativas. En vista de estos resultados, se estudió la estabilidad temporal de la respuesta de los vasos primaverales a la NAO durante el período 1825-2003, tratándose de una señal no completamente estable y con dos patrones diferentes. HYD-r1 mostró una tendencia inicial ascendente para luego descender con rapidez, siendo la correlación no significativa durante buena parte del siglo XX, y con una ligera mejoría en las últimas décadas, mientras que la tendencia de HYD-nr1 fue descendente desde el principio, alcanzando valores no significativos entre 1895-1930 pero recuperando muy rápidamente los valores a partir de ese momento, hasta alcanzar correlaciones similares a las del inicio.

Esta cronología de 481 años es la más larga obtenida hasta el momento mediante el empleo de vasos de madera temprana, y arroja unos resultados prometedores de cara al empleo de características de los vasos como *proxy* climático. Se apreció que su respuesta al clima no parece ser dependiente de la edad, y mostró una alta correlación con el clima durante un considerable número de años.

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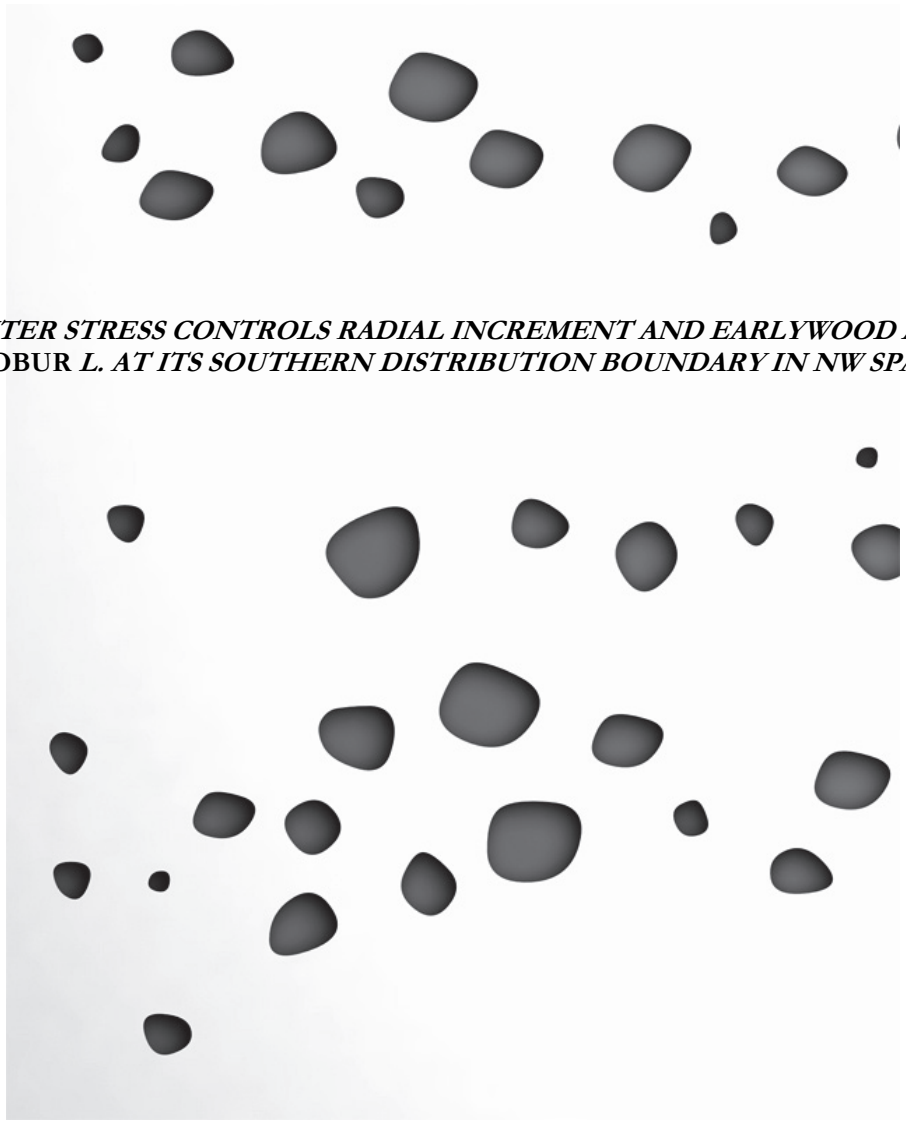

ARTICLE I. WINTER STRESS CONTROLS RADIAL INCREMENT AND EARLYWOOD ANATOMY OF* QUERCUS ROBUR *L. AT ITS SOUTHERN DISTRIBUTION BOUNDARY IN NW SPAIN

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Abstract

We selected two stands dominated by *Quercus robur* L. in the northwestern Iberian Peninsula, close to its distribution limit to the Mediterranean region, which only differed in their water-holding capacity. We obtained tree-ring chronologies using radial increment and earlywood anatomy, and established climate-growth relationships for the period 1956-2013. The study variables were the mean area of the earlywood vessels separating those in the first row (MVA-r1) from the rest (MVA-nr1), and the latewood width (LW).

Our results showed that earlywood anatomy was strongly controlled by conditions during the dormant period, whereby an elevated winter temperature appears to induce a high consumption of carbohydrates, resulting in smaller vessels. In addition, this effect was reinforced by waterlogging at the wettest site, which also showed a high correlation to winter precipitation. The different soil water regime induced differences in vessel rows, since all earlywood vessels were controlled by winter conditions at the wettest site, but only the first row at the driest one. Radial increment was not related to water availability during the growing season, but to the previous year.

We hypothesize that *Q. robur* trees growing close to their southern distribution boundary adapt a conservative strategy, prioritizing reserve storage under limiting conditions during the growing period. As a results, wood formation is mainly controlled by the balance between the accumulation of carbohydrates during the previous season, and its consumption to maintain respiration during dormancy and early growth in spring.

Keywords: dendrochronology, tree ring, quantitative wood anatomy, earlywood vessels, waterlogging, distribution boundary

Introduction

Transition zones between large biogeographical boundaries are of great ecological interest, because they act as bridges for the movement of taxa during environmental changes (Sánchez-de-Dios et al. 2009). This is the case of the Mediterranean region in southern Europe, where climatic predictions indicate a tendency to increase moisture deficit during the warm season, severely impacting vegetation (IPCC 2013). The Iberian Peninsula is a transition territory of greatest importance, because many temperate species reach their southernmost geographical frontier, and are progressively replaced by more drought-tolerant species, as happens with the nemoral oak species *Quercus robur* and *Q. petraea* (Sánchez-de-Dios et al. 2009). In fact, future climatic models predict a considerable reduction of the area occupied but these oaks, which would be even more if habitat fragmentation is considered (Benito Garzon et al. 2008). The northwestern Iberian Peninsula constitute one of these boundary areas, as many small patches of *Q. robur* woodlands are the dominant natural forests under Atlantic climatic regime, but they suffer from a moderate summer drought, being progressively replaced by Mediterranean formations towards the inland.

Tree species growing in boundary areas suffer from different sources of stress, among which summer drought has been pointed out as one of the main potential limitations for growth, often resulting in increasing mortality (Allen et al. 2010; Camarero et al. 2015; McDowell et al. 2008). However, a higher water demand, either caused by a reduction in rainfall, or the increment of temperature and thus evapotranspiration, is not the only climatic stress trees can face in temperate regions. High amounts of precipitation associated to a relatively high temperature in winter have demonstrated to be the main cause for oak dieback in northwestern Spain (Rozas and García-González 2012b). In fact, temperate oak species such may be suffering from a 'double stress' in this region, considering this as the combining effect of summer and winter conditions. *Q. robur* is a ring-porous species, i.e., with a strategy consisting in forming large earlywood vessels for a rapid development in spring when water is highly available, and much smaller vessels during water shortage in summer (Aloni 2015). But the large earlywood vessels are in general functional for a single growing

season (Cochard and Tyree 1990), and the first elements are formed before bud break (Pérez-de-Lis et al. 2015; Takahashi et al. 2013), so that they must be supported by reserves accumulated during the previous growing season (Gallé et al. 2007). Therefore, trees need to maintain a balance between carbohydrate storage during the growing season, and its consumption during dormancy and spring reactivation.

Tree-ring analyses are one of the most commonly used methods to study the responses of trees to environmental conditions, and it is especially useful if combined with the variations in xylem anatomy (Fonti et al. 2010). The study of earlywood vessels has proved to be successful to the study the behavior of sympatric oaks growing close to their distribution boundary in northern Spain (González-González et al. 2014; González-González et al. 2015), or to understand the relation to climate for several Mediterranean oaks (Corcuera et al. 2004; Corcuera et al. 2006; Gea-Izquierdo et al. 2012). In addition, it proved to be suitable for mesic areas, without the existence of a prevailing climatic factor (Fonti and García-González 2008). The variation in xylem architecture is directly linked to hydraulic properties of the tree (Fonti et al. 2010; Gea-Izquierdo et al. 2012), yielding a proxy of a great ecophysiological relevance (González-González et al. 2014), which is commonly rather different from tree-ring width (Fonti and García-González 2008; García-González and Eckstein 2003; González-González et al. 2015). Consequently, the analysis of both radial increment and earlywood anatomy are probably the best approach to understand the effect of climate on oak performance, as both tree-ring compartments are rather different from each other.

Although water availability is one of the most important factors in terrestrial ecosystems, the study of its impact on tree species by using time series for several decades is not always straightforward. Precipitation records are not always reliable for a given region, especially if rainfall is highly modified by orography. In addition, water available for the plants is also controlled by evapotranspiration, which is highly dependent of temperature and vegetation cover, and soil properties. One possible approach is to compute water balance as the difference between precipitation and evapotranspiration (Bigler et al. 2006; Rozas et al. 2009), or even soil water estimates (González-González et al. 2015). The Palmer's Drought Severity Index, known as PDSI (Palmer 1965), has been commonly used in dendrochronology, but it has limited utility in areas other than those used for calibration. In contrast, the Standardized Precipitation-Evapotranspiration Index (SPEI), defined by Vicente-Serrano et al. (2010b) overcomes some of these limitations, and has been successfully used to determine the vulnerability of forests to drought stress at a global scale (Vicente-Serrano et al. 2014). In fact, a correct analysis of the effect of water regime should involve droughts as a function of time scale (Vicente-Serrano et al. 2013), i.e., the time lag between the onset of water shortage and the identification of its consequences on growth (Vicente-Serrano et al. 2014).

In this paper, we analyze the effects of climate on wood anatomy and radial increment of *Quercus robur*, growing close to the limit of its distribution boundary towards the Mediterranean regions in NW Spain. For this, we selected two stands representative of the most common forest patches existing nowadays in the area, which differ in their soil water regime. We test if climate controls wood formation of *Q. robur* at its southern distribution boundary by operating through two different sources of stress in the active and dormant periods, respectively. Specifically, we hypothesize that i) a low water availability constraints growth by limiting assimilation during summer time, and ii) a high winter temperature increases respiration and impacts early growth by reducing available carbohydrates.

Materials and Methods

Study area and sites

The study area is located within the southern distribution boundary of *Quercus robur* in NW Spain, on a basin around 500 m asl., and

surrounded by gentle hills that do not exceed 900 m asl. (Fig. 1; Table 1). The area is characterized by a subhumid Atlantic climate with a strong seasonality; moderate rainfall (800 mm yr^{-1}) is mainly concentrated in autumn and winter, and is lower than general precipitation in most of the region, which reach values of 950 mm yr^{-1}. Summers are warm (mean temperate higher than 20º C in July and August), with a low precipitation, which results in a drought period of at least these two months. Winter temperature is relatively mild (around 7º C in January and February), but the occurrence of late frost is common due to the inland location. Although these sites are not situated within the Mediterranean Region, the boundary to this biogeographical territory elapses very close to the sampled sites (Rivas-Martínez 1987).

We sampled two natural nearby stands dominated by *Q. robur*, but on different topographical positions (Fig. 1). Both sites can be considered subhumid Atlantic oak forests belonging to the association *Rusco aculeati-Quercetum roboris* (Amigo et al. 1998; Rivas-Martínez 1987). Such woodland type is characterized by the dominance of oaks (namely *Q. robur*, but *Q. pyrenaica* can often be common in the canopy), and by the lack of understory species indicating moist conditions that are common in other oak forests in NW Iberia. In contrast, Mediterranean or thermophile elements usually occur. Woody species such as *Laurus nobilis* L. or *Arbutus unedo* L., together with *Ruscus aculeatus* L. or *Tamus communis* L. are usually present in these forests; other common accompanying species included *Holcus mollis* L. or *Lonicera periclymenum* L.

Ramirás (RAM) is a little and monospecific woodland located at 450 m asl., on a gentle slope facing north where watercourse runs seasonally, and surrounded by meadows. The largest trees have long and straight stems, and are regularly distributed throughout the stand. The soil was deep, and water availability appears to be high for a long period of the season, in general much more than usual within the surrounding region.

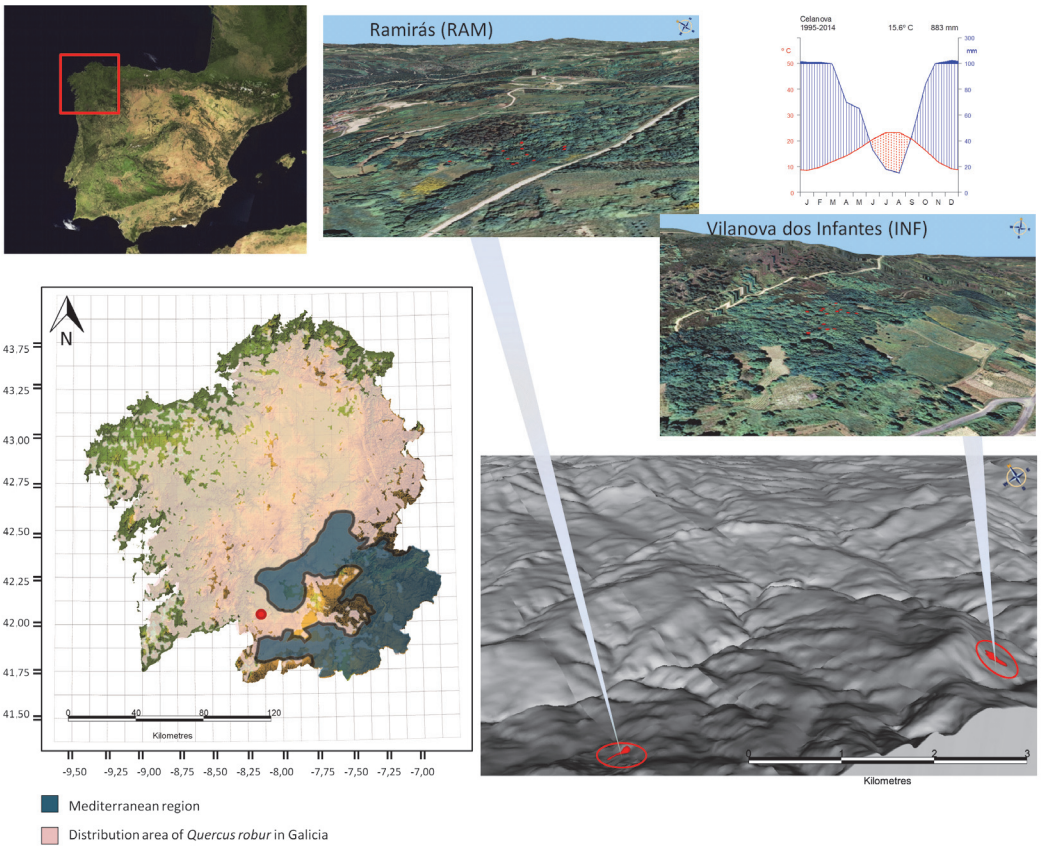

Fig. 1. Location of the two stands in the southern distribution boundary of *Quercus robur* in Galicia. A Digital Terrain Model (DTM) of each plot is shown, with sampled trees as red points, and climate diagram from Celanova weather station.

Table 1. Physiographic characterization of the study sites.

Location	Code	Area (ha)	Elevation (m)	Facing	Slope (%)	North Latitude (º)	West Longitude (º)
Ramirás	**RAM**	1.2	450	North	5-10	42.17	8.00
Vilanova dos Infantes	**INF**	2.7	480	South	15-30	42.17	7.95

Laurus nobilis trees commonly occur under the main canopy, together with other thermophile understory species as *Ruscus aculeatus*, and several fern species s associated to water streams. This forest had a mixed use in the past, i.e., it served as pastureland for cattle during summer time, and also for fuelwood and timber extraction.

Vilanova dos Infantes (INF) is also a pure *Q. robur* forest situated on a southern moderate slope at 480 m asl., as the result of abandonment of ancient farmlands. Old trees are frequent and regularly distributed throughout plot, usually originating from oldest pollarded oaks that constituted the boundary lines between contiguous fields. Floristic composition is very similar to RAM, with the thermophile presence of *Ruscus aculeatus* as understory species, but without *Laurus nobilis*, and a considerably scarcity of ferns. In addition, the invasive *Acacia dealbata* Link. occurs at the edge of the forest and in disturbed areas, evidencing more human pressure than at the former site.

Sampling, processing and anatomical measurements

We selected 14 dominant trees at RAM and 15 at INF for sampling, and extracted at least two 5-mm cores from each tree, using an increment borer at breast height. Cores were air-dried, glued onto wooden supports, and prepared for an optimal visualization of the cross-sectional surface. We obtained a regular surface by means of a WSL sliding microtome (Gärtner and Nievergelt 2010), followed by manual polishing with progressively finer sandpaper (grain sizes from P220 to P1200, FEPA (Federation of European Producers of Abrasives). Vessel lumina were cleaned from accumulated wood dust and tyloses using high-pressure water blast (Fonti et al. 2002). We finally stained the samples with black printer ink, and the earlywood vessels were filled with chalk dust (González-González et al. 2014) to gain an optimal contrast for image analysis.

We measured earlywood width (EW) and latewood width (LW) to the nearest 0.001 mm, using an appliance composed by a tree-ring measuring linear stage (Velmex TA UniSlide, Velmex Inc., Bloomfield NY, USA) implemented with a binocular microscope (Olympus SZ60) at 20-40x magnification. The boundary between earlywood and latewood corresponded to differences on vessel size and in wood structure (García-González and Eckstein 2003). Earlywood conductive elements are much larger, and disposed in a continuous band, whereas the smaller latewood vessels are in flamelike groups along radial strips. For crossdating, we visually compared individual curves of total ring width (RW), calculated as the sum of EW and LW, and we statistically verified the accuracy of dating using COFECHA (Grissino-Mayer 2001).

We chose a subset of 25 trees (12 from RAM, and 13 from INF) for vessel measurements. The selected trees did not exhibit breaks, anomalies, or unsure dating, and were highly correlated to the local chronology. The cores were sequentially digitized using a device composed by a digital camera Canon EOS 600D, coupled to a binocular microscope Olympus SZ60. Each sample was automatically moved along a mechanically driven platform in order to obtain consecutive high-resolution images (5184 x 3456, 17.9 Mpx) from the core surface. Pictures from the same core were stitched into a single image using PTGui ver. 9.1.8 Pro (New House Internet Services B.V., Rotterdam, The Netherlands), which was saved into a Tagged Image File Format (TIFF) file to avoid loss of quality. Images were analyzed in ImageJ (Abramoff et al. 2004; Schneider et al.

2012) using the VesselJ plugin (García-González, not published), and earlywood vessels were automatically recognized and measured on a 8-bit (gray) threshold level due to contrast differences between the dark background tissue and the white vessel lumina. We automatically removed objects larger than 10,000 μm^2 or twice longer than wide by the so-called size and shape filters, whereas other undesired objects detected as vessels, or incomplete vessel outlines, had to be corrected manually. The application of the convex hull, and a morphological operation (erode-dilate 2x2 one pass) optimized vessel shape before storing the values.

Due to the fact that the whole core was analyzed as a single image, we had to assign each vessel to the correct dated ring after image analysis. For this, we used the self-develop program Autovasos (García González, not published), which allowed us to detect and modify tree-ring boundaries along with their corresponding earlywood vessels, as well as to cross-check the rings detected on the core images against the dated RW series. During vessel assignment, we also separated the first row of vessels from the others, as recommended by García-González and Fonti (2008). All vessels located immediately after the ring boundary, and those initiating not farther than the imaginary line connecting the centroids of boundary vessels, were considered to belong to the first row.

Chronology building and assessment

We obtained chronologies on a tree basis, i.e., width measurements from all cores of the same tree were averaged, and all vessels belonging to the same ring were pooled. Afterwards, we calculated the mean area of all vessels (MVA) for each ring, but discriminating between vessels in the first row (***r1***), and those not located in this row (***nr1***), as they are formed at different moments of the growing season, and potentially controlled by different driving factors. Latewood width (LW) was also included in the analyses to better evaluate radial increment.

Growth-related trends were removed from the series by adjusting a 32-year cubic smoothing spline (Cook et al. 1992) with a 50% variance reduction frequency. This spline function is flexible enough to remove age- and disturbance-related trends, while adapting to both the ascending trend of anatomical variables and the descending trend of latewood. The division of each measured value by the parameter estimated by the function yielded dimensionless growth indices (Fritts 1976), and we averaged them into a mean chronology using a biweight robust mean (Mosteller and Tukey 1977).

We finally obtained six chronologies (MVA-r1, MVA-nr1, and LW at both sites), and we carried out all subsequent analyses along a common interval 1956-2013 (58 years) covered by data from at least 11 trees per site. We assessed the statistical quality of all series using descriptive statistics, as the mean sensitivity (*MS*) to evaluate year-to-year variability, and the first order autocorrelation coefficient (*AR1*) to estimate the influence of prior growth. Common signal of chronologies was evaluated by means of several coefficients commonly used in dendrochronology (Cook et al. 1992), namely the mean correlation between trees (*Rbt*), the signal to noise ratio (*SNR*), and the expressed population signal (*EPS*). *Rbt* is the mean value of all possible Pearson's cross-correlation coefficients, *SNR* is the resulting value from the division between *Rbt* and the statistical amount relative to noise, and *EPS* indicates the extent to which the sample size is representative of a theoretical population with an infinite number of individuals (Wigley et al. 1984). The former is independent of sample size, whereas the other two are strongly influenced by the number of trees, especially if this is low. We also compared variable chronologies between sites by calculating their Pearson's cross correlation.

Climate-growth relationships

We established correlations between the chronologies and climatic data (meteorological records and a drought index) for the period 1956-2013 (58 years). Although we used simple Pearson's correlations, their significance was assessed by 10,000 iterations of the bootstrap technique (Guiot 1991) for each coefficient, and

applying the correction proposed by Mason and Mimmack (1992) for the percentile bootstrap confidence intervals. Additionally, correlations functions were also computed on single trees, as individual responses can sometimes provide valuable additional information (Rozas 2015).

Series of temperature and precipitation were obtained from the gridded data source CRU TS 3.22, available in KNMI Climate Explorer (http://climexp.knmi.nl/), at 0.5º of spatial resolution, and covering the period 1901-2013. We also calculated the Standardized Precipitation-Evapotranspiration Index (SPEI), a multiscalar drought estimator that involves water balance (precipitation minus potential evapotranspiration), the cumulative water deficit/surplus at different time scales, and the adjustment to a log-logistic probability distribution (Vicente-Serrano et al. 2010a). The SPEI has additional advantages in relation to previous indices, such us the self-calibrated Palmer's Drought Severity Index (PDSI), and has been successfully used to study the responses of vegetation growth to drought (Vicente-Serrano et al. 2012). Values for the index were calculated in R (R Development Core Team 2013), using the package 'SPEI', and using time scales for 1 to 21 months.

Climate-growth relationships were studied along the seasonal period that could apparently have some influence upon the growing season. This comprised from previous to current May (13 months) for MVA-r1 y MVA-nr1, and to current October (18 months) for LW; relations between these variables and SPEI were analyzed until December (20 months). In addition, we seasonalized monthly values, considering dormancy-quiescence (December-February) for MVA series; and early spring (April-May) and early summer (June-July) for LW, in order to better understand the relationships between climate and growth.

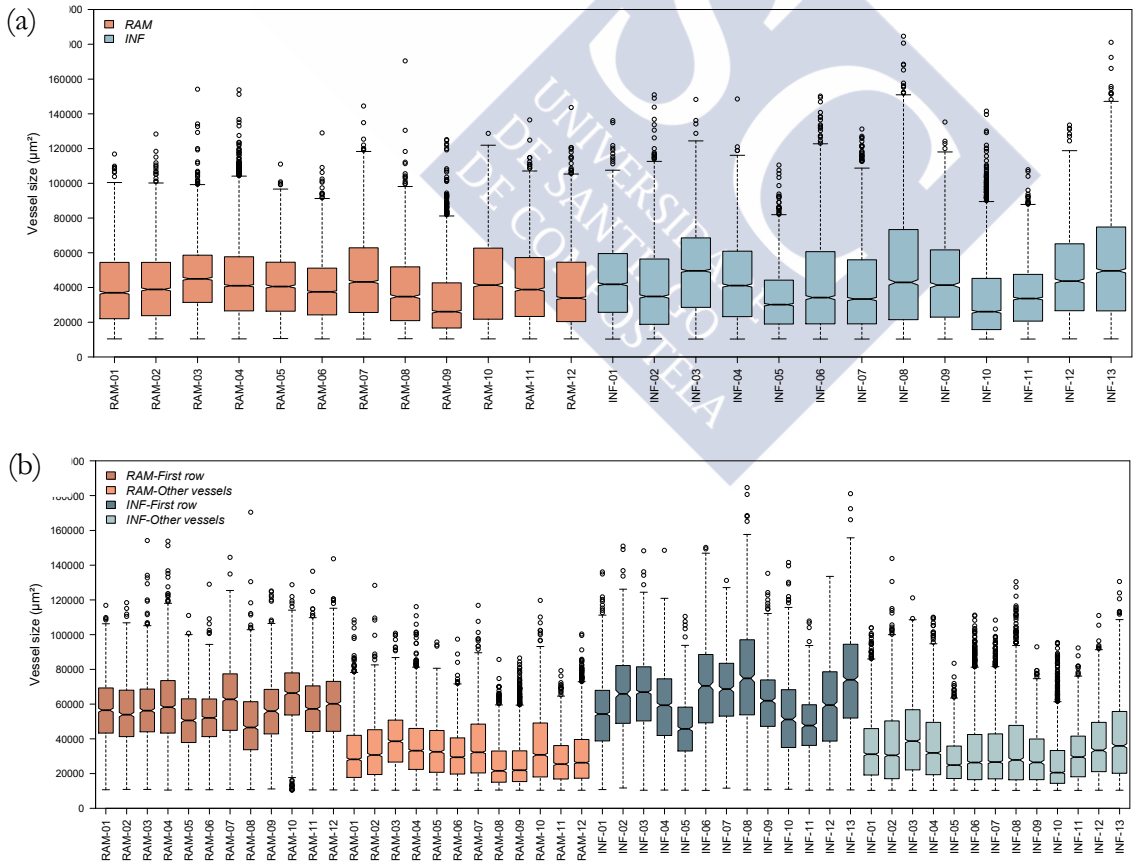

Fig. 2. Boxplots representing the distribution of total number of measured vessels (a), and separated by rows (b) for both sites.

Table 2. Descriptive statistics of vessel distribution at Ramirás (RAM) and Vilanova dos Infantes (INF), for total ring width (italics), and separating between first row (r1), and vessels not in this row (nr1). *N*: number of vessels; *Max*: Maximum vessel area in μm²; *Average*: Mean vessel area in μm²; *SD*: Standard deviation; *CV*: Coefficient of variation; *Skew*: Skewness.

Location	Row	N	% vessels	Max	Average	SD	CV	Skew
	All	*46269*	*100*	*170471.0*	*41211.7*	*21531.4*	*0.52*	*0.63*
RAM	r1	16768	36.2	170471.0	56594.3	20569.3	0.36	0.18
	nr1	29501	63.8	128303.6	32468.5	16604.0	0.51	0.82
	All	*46488*	*100*	*184661.7*	*43014.1*	*25275.3*	*0.59*	*0.81*
INF	r1	15295	32.9	184661.7	61332.8	25587.7	0.42	0.34
	nr1	31193	67.1	143772.3	34031.8	19642.7	0.58	0.96

Results

Vessel-size distribution and comparison between chronologies

We measured a comparable number of vessels at each site (46,269 vs. 46,488). Vessels were larger at INF (43,014 μm^2 vs. 41,212 μm^2), which was clearer when comparing the first rows (61,333 μm^2 vs. 56,594 μm^2), and less evident for the rest of earlywood vessels (34,032 μm^2 vs. 32,469 μm^2) (Fig. 2, Table 2). Size distribution was skewed towards the smallest vessels at both sites, mainly caused by vessels not in the first row, which also presented greater amount of anomalous values (Fig. 2).

LW chronologies showed a better statistical quality than MVA (Table 3), with mean correlation higher than 0.35, and EPS higher than 0.85 in both cases, the minimum value recommended by Wigley et al. (1984). Chronology quality was considerably lower for MVA, especially for MVA-nr1, but the values for both rows did not differ or were even higher than those successfully used for dendrochronological analysis of anatomical variables ((Gea-Izquierdo et al. 2012; González-González et al. 2014; Hroš and Vavrčík 2014)). *SNR* and *EPS* values showed the same pattern.

Year-to-year variability, expressed as *MS* values, was very low for MVA (0.08-0.09), and much higher for LW (0.28-0.33); in addition, LW clearly reflected abrupt growth changes that did not impact vessel size. Autocorrelation was negligible at RAM, and slightly higher at INF mainly for MVA-r1, but still with low values. As regards differences between sites, INF showed the best statistics for LW, whereas RAM did for both earlywood variables.

Table 3. Descriptive statistics of the chronologies at Ramirás (RAM) and Vilanova dos Infantes (INF), for the common interval 1956-2013. Total covered period was 1890-2013 (124 years) at RAM, and 1840-2013 (174 years) at INF. Variables considered were MVA-r1 (mean area of vessels in the first row), MVA-nr1 (mean area of vessels not in the first row), and LW (latewood width). *Rbt*: mean correlation between trees; *SNR*: signal-to-noise ratio; *EPS*: expressed population signal; *MS*: mean sensitivity; *AR1*: variance in the first eigenvector.

Chrono	MVA-r1		MVA-nr1		LW	
Plot	**RAM**	**INF**	**RAM**	**INF**	**RAM**	**INF**
Rbt	0.30	0.21	0.21	0.19	0.37	0.44
SNR	4.75	2.89	2.59	2.39	6.36	8.57
EPS	0.83	0.74	0.72	0.70	0.86	0.90
MS	0.09	0.08	0.08	0.09	0.28	0.33
AR1	0.05	0.38	0.10	0.27	0.20	0.33

Both sites were close to each other, and consequently correlations between them were consideraby high (Fig. 3). The common pattern was clearer for LW (r=0.74) and MVA-r1 (r=0.70) than for MVA-nr1 (r=0.58). Therefore, the information provided by the first two variables is expected to be very similar for both stands, while the greatest differences should be recorded by MVA-nr1. When comparing variables within sites, we found some differences between RAM and INF. At RAM, LW was not correlated to MVA regardless of the row considered, but both earlywood variables were rather similar (r=0.72, P<0.0001),

indicating that earlywood and latewood accounted for a different kind of information.

Table 4. Correlations between variable chronologies at each site for the study period. MVA-r1: mean area of vessels in the first row; MVA-nr1: mean area of vessels not in the first row; LW: latewood width. Non-significant values are designated as *n.s.*

Correlation	RAM	INF
MVA-r1 / LW	0.05 / n.s	-0.06 / n.s
MVA-nr1 / LW	-0.48 / p<0.0001	-0.17 / n.s
MVA-r1 / MVA-nr1	0.72 / p<1x10^{-9}	0.51 / p<0.0001

In contrast, correlation between MVA-r1 and MVA-nr1 was lower at INF (r=0.51, P<0.0001), whereas LW was negatively correlated to MVA-nr1, i.e., smaller vessels at the end of earlywood were coupled to wider rings; similarly to RAM, MVA-r1 and LW seem to be independent from each other (Table 4).

Climate growth-relationships

MVA-r1 and MVA-nr1 showed strong relationships to climate (Fig. 4). Differences were also remarkable between sites, especially when comparing the pattern of responses between vessel rows. MVA-r1 was highly correlated to conditions during dormancy, especially when the period for analysis was previous December to current February. Temperature exhibited a highly negative correlation to vessel size, which was maximized by minimum temperature (r=–0.59, P<0.0001 for RAM; r=–0.51, P<0.0001 for INF), but completely absent with maximum temperature. However, sites differed in relation to precipitation, as the wettest site RAM was much more correlated than INF (r=–0.60, P<0.0001 vs. r=–0.38, P<0.01). But both sites had a different behavior not only as regards the role of winter precipitation, but also with the climatic response of MVA-nr1. While relationships at RAM appeared to be a somehow weaker continuation of those observed for MVA-r1, there was a completely different signal for INF, as *nr1*-vessels were positively related to temperature in May (r=0.45, P<0.001), and negatively to precipitation (r=–0.35, P<0.01), i.e, a warm and/or dry spring should be associated to larger vessels; a certain relation to temperature in the previous summer was also observed (r=0.30, P<0.05).

LW registered less significant correlations with climate than MVA (Fig. 5), evidencing a weak response of latewood to climate. Both sites had a similar pattern, as they showed some negative relation to temperature in the previous summer (r=–0.31, P<0.01 at RAM; r=–0.33, P<0.01 at INF), and also the positive effect of early spring (April) precipitation (r=0.30, P<0.01 at RAM; r=0.30, P<0.01 at INF). In contrast, conditions during the end of the previous season and dormancy were more remarkable for INF, especially with regard to the effect of rainfall during previous November (r=0.37, P <0.001); there was also a clear relationship between precipitation in September and LW (r=–0.37, P <0.001) for INF, which could be indicating the existence of cambial reactivation for a short period of wood formation in autumn.

A great extent of the differences that arose between sites are probably due to the constrasting soil water regime at both sites, and this effect is better described when using SPEI, as the values of this index are indicative of the magnitude and persistence of soil water excess/deficit. Thus, correlations between MVA and SPEI showed sharp differences in the intensity and length of the detrimental effects of waterlogging episodes, in the subset of vessels affected, and in the length of the sensitive period at each site (Fig. 6). We found higher negative correlations for MVA-r1 at RAM, with markedly significant values at time scales from 1 to 19 months. The period of greatest influence comprised from previous December to current July, with a maximum in March, corresponding to a lag of six months. MVA-r1 was hardly influenced at RAM. However, it was MVA-nr1 and not MVA-r1 that was related to SPEI at INF. Correlations were highly significant throughout shorter time scales (from 8 to 18 months), and only for April-September, with a maximum in current May for a lag of 14 months.

The response pattern for LW was similar for both sites, with a positive correlation at the same time of growing season (Fig. 7), probably

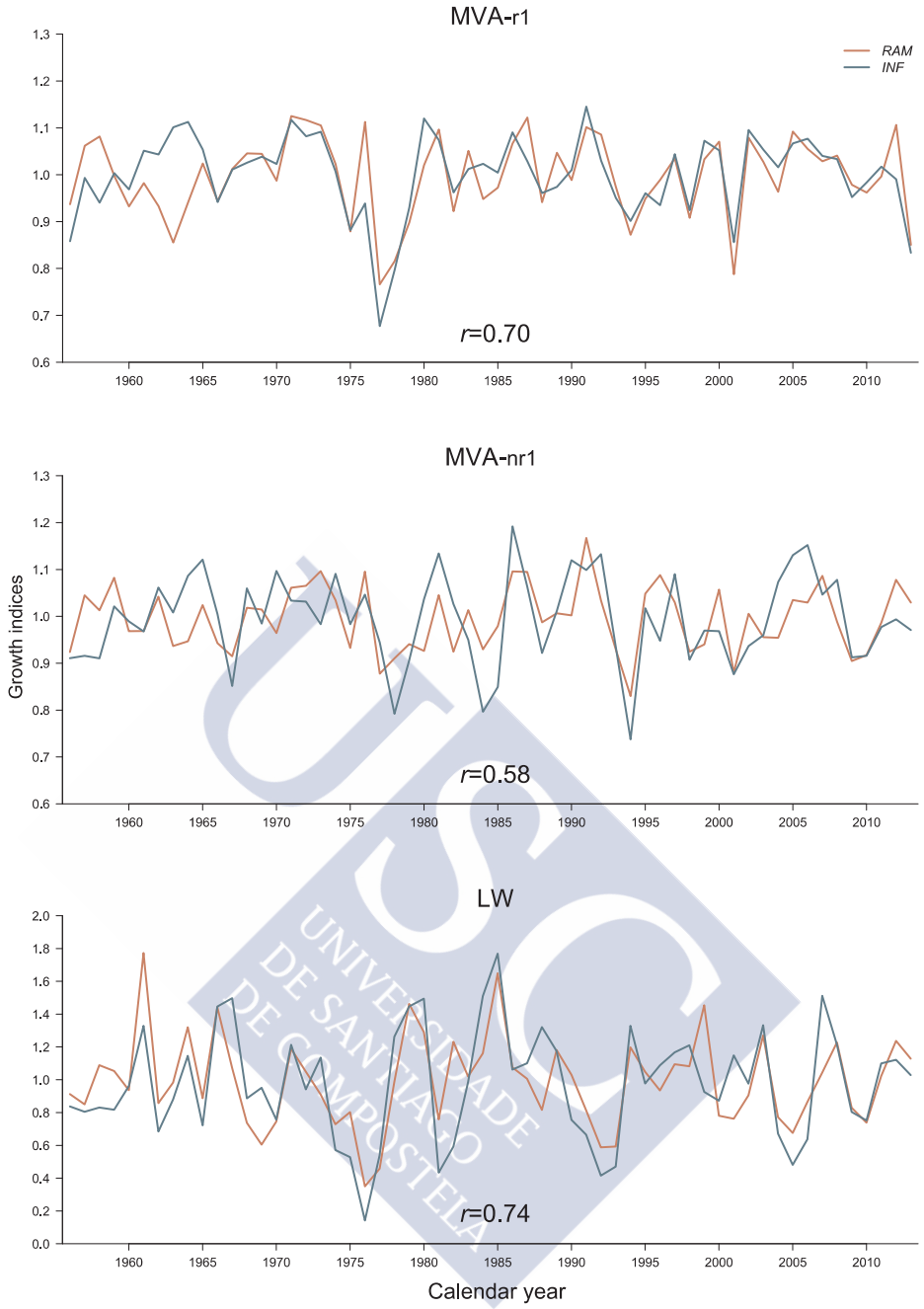

Fig. 3. Comparison between both site chronologies, for mean vessel area of the first of row vessels (MVA-r1), vessels not in the first row (MVA-nr1), and latewood width (LW), along the common interval 1956-2013.

related to the conditions in the previous year. But correlations were higher and for a longer period at the driest site INF, maximizing in current early summer, at lags of 18 and 19 months (May and June). In contrast, the highest correlation at RAM corresponded to April for a time lag of 19 months. The weak effect of late summer conditions at INF was also registered for current September at a time scale of two months.

The adjustment between earlywood variables and the monthly SPEI series for the time scales maximizing their influence of vessel formation (March for MVA-r1 at RAM, and May for MVA-nr1 at INF) supports the results found for anatomical variables, as the agreement is

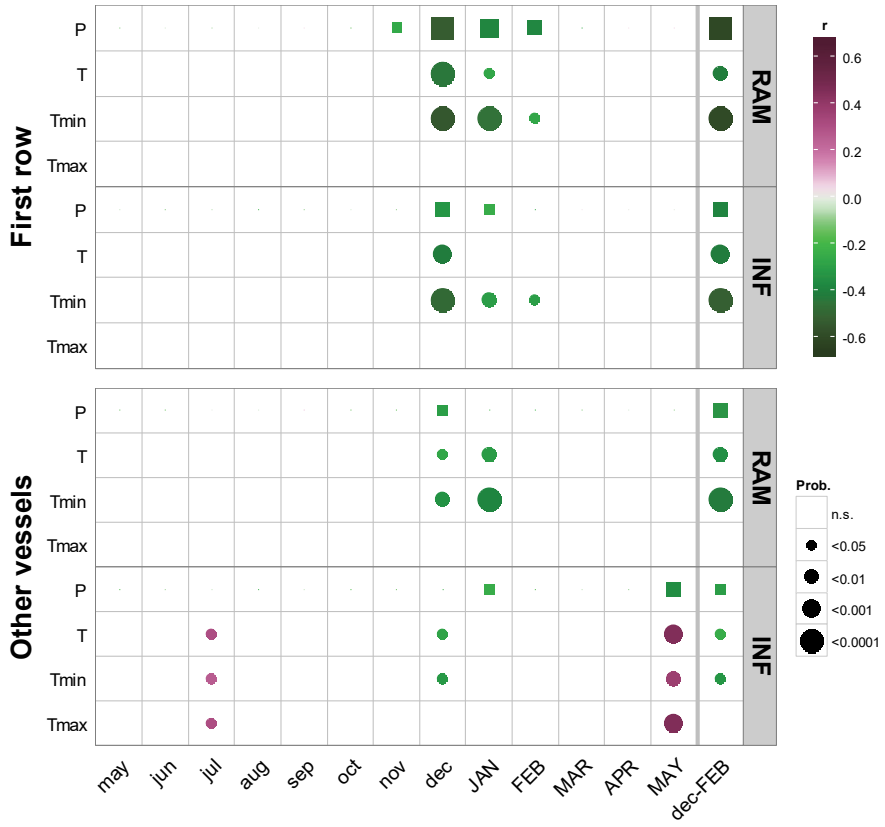

Fig. 4. Relationships to climate for mean vessel area (MVA), expressed as bootstrapped correlation coefficient, along the study period 1956-2013; vessel rows were considered separately. Lower and uppercase letters correspond respectively to the months of the previous and current growth years.

clear along the whole series (Fig. 8). As regards LW, this adjustment shows the clear relation between radial growth and water availability in spring-early summer, evidencing a direct dependence on conditions of the current growing season (Fig. 8).

Finally, the divergent role of conditions during the dormant season on vessel size can also be observed when analyzing the individual responses of trees (Fig. 9). For MVA-r1, ten out of 12 trees (83 %) had the same relationship as the mean chronology to both temperature and precipitation at RAM, whereas only eight out of 13 trees (62 %) were related to temperature at INF, and just six to precipitation (46 %). The predominace of single responses for MVA-nr1 was evident during this period, but also in May when only seven out of 13 trees (54 %), and four out of 13 trees (31 %) collected the signal of temperature and precipitation at INF.

Discussion

Preliminary considerations on variables and sites

We studied two nearby sites, where *Q. robur* was growing close to the southern boundary of this geographical distribution, under the same climatic conditions, and only differing in their water holding capacity. Consequently, chronologies between sites were highly correlated for each variable, but this similarity was highest for LW, and much lower for MVA-r1. When comparing variables within sites, LW and MVA-r1 were independent, LW correlated to MVA-nr1 only at the wettest site RAM, and also vessel rows were more similar to each other at this site. These results indicate that i) differences between both tree-ring compartments (earlywood and latewood) are remarkable, and ii) these differences are context

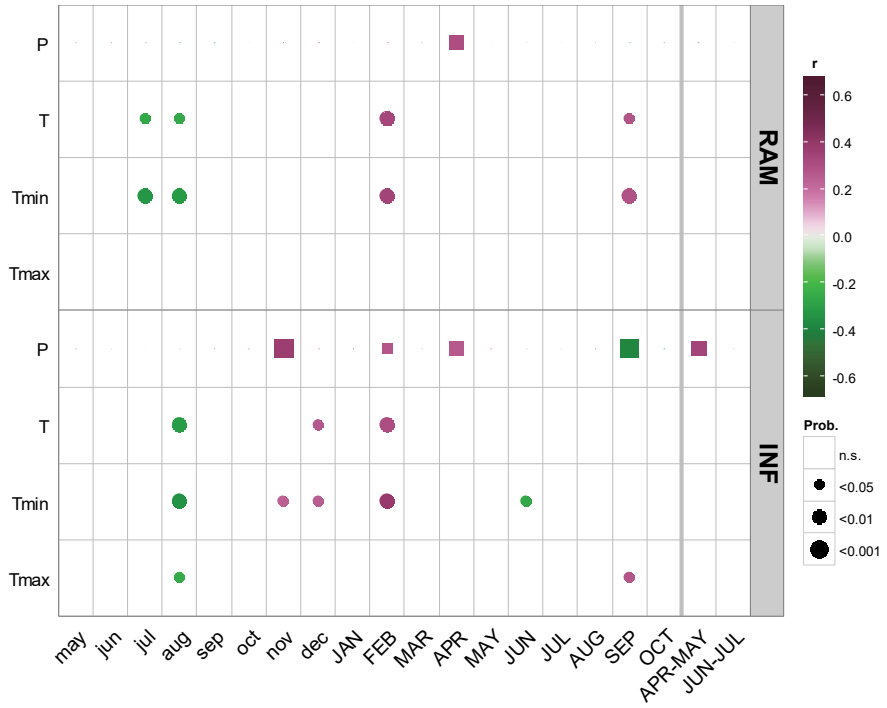

Fig. 5. Relationships to climate for latewood width (LW), expressed as bootstrapped correlation coefficient, along the study period 1956-2013. Lower and uppercase letters correspond respectively to the months of the previous and current growth years.

dependent, of greater or lower importance depending on the site.

One of the main reasons to use earlywood anatomical variables is that they usually provide another type of information than that recorded by ring width (Fonti and García-González 2004; Fonti et al. 2010; García-González and Eckstein 2003; González-González et al. 2014; Kniesel et al. 2015). The separate analysis of both parts of the ring provides consequently robust information on the climatic control of wood formation that could not be shown using 'classical' dendrochronology. As a result, earlywood vessels of ring-porous trees seem to be a promising proxy in areas where a prevailing limiting factor is not operating (Fonti and García-González 2008; Fonti et al. 2007). But the (lack of) association between earlywood anatomy and latewood is not the same for all cases or species, although no research has been specifically focused on understanding this relationship. However, a few results point out that the differences between both anatomical compartments probably tend to be reduced if there is a strong limiting factor, as e.g., towards xeric environments. Thus, Fonti and García-González (2008) observed that earlywood vessels of oak species had a strong climatic signal along a gradient of decreasing precipitation, but their relationship was similar to that found in the ring width for the sub-Mediterranean *Q. pubescens*, which grew under much drier conditions. Similarly, Campelo et al. (2010) found a strong relationship between winter-spring precipitation and both tree-ring width and maximum vessel area of the diffuse-porous *Q. ilex*, growing in the southwestern Iberian Peninsula.

In view of our results, a stronger climatic control appears to be linked to more similarity among growth variables. The wet site RAM is apparently the most limited by environmental conditions, which results in a more intense, but also more uniform, response to climate. This explains why MVA-nr1 and LW were only associated at this site, and why both rows of earlywood were highly correlated, and controlled by conditions during dormancy, whereas MVA-nr1 was related to late spring at INF. Moreover, although differences between sites with regard to overall vessel size were scarce, the driest INF had moderately larger

vessels, and more variations among individuals; but differences were only evident for the first row of vessels. Consequently, trees at the driest site were slightly more 'ring-porous', i.e., exhibited more differences between the largest and the smallest earlywood vessels. Ring porosity has been described as an adaptation to temperate ecosystems with a clear contrast between seasons (Aloni 2015). This is compatible with the fact that trees at INF showed more range of variation in vessel size, as this site has more variation in water availability along the season. But this idea should be taken with caution, because differences were not notable, and there are many other factors than only climate influencing vessel size. In fact, Astrade and Begin (1997) found oak tree rings with a wide earlywood area, resembling a semi-ring porous structure, and related them to flooding markers; however Gričar et al. (2013) stated that earlywood anatomy was more affected by ring width than by hydrological conditions.

The differences observed between rows are also of methodological importance, because one of the main questions when analyzing the earlywood vessels of ring-porous trees choose among a large pool of many potential variables. This has been performed by either an *a priori* selection, usually by means of PCA (Fonti et al. 2007; Kniesel et al. 2015), or *a posteriori* in view of climate-growth relationships (Fonti and García-González 2004; González-González et al. 2014). Among this decision, the use of the whole vessel data set or to split it into rows is fundamental, because vessels are formed at different times of the season, and may provide another kind of information (García-González and Fonti 2006). According to our results, the answer is not straightforward, because we showed that additional information could be

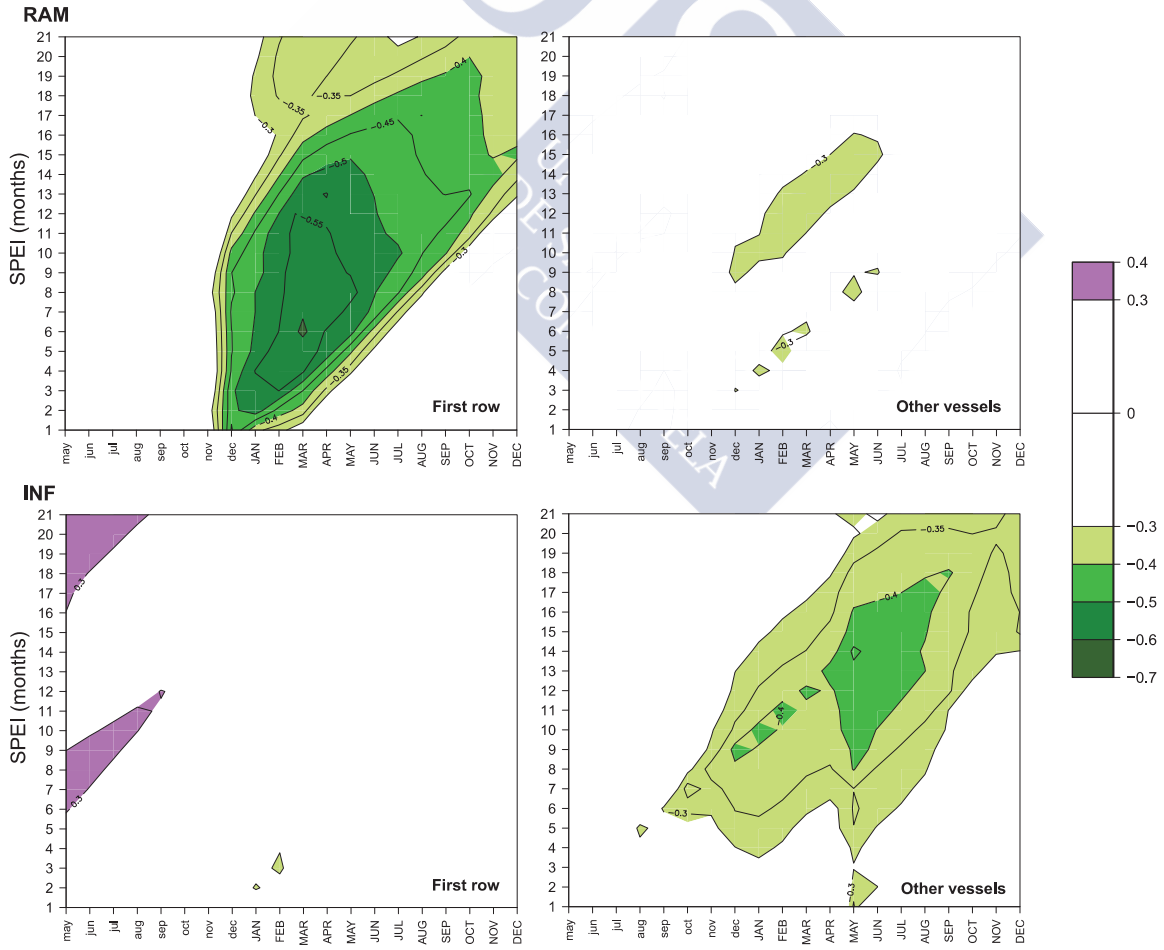

Fig. 6. Correlations between MVA site chronologies separated by vessel rows and monthly SPEI values at different time scales (1-21) for the study period 1956-2013. Lower and uppercase letters correspond respectively to the months of the previous and current growth years.

gained by splitting vessels at the driest site INF, but not at RAM, where winter conditions were probably more limiting, and consequently most vessels controlled by the same factor.

Effects of climate on wood anatomy and radial increment

Climate-growth relationships showed a strong influence of conditions during the dormant season on the size of the earlywood vessels at both sites. There was a very significant negative correlation between temperature (mean and minimum) in winter and the size of the earlywood vessels, whereby a warm winter is coupled to smaller vessels, especially to those found in the first row. Consequently, we hypothesize that a high temperature in winter causes a considerable degradation of stored carbohydrates to maintain respiration, which in turn influences vessel size in the following spring. Furthermore, we believe that it is the dormant period and not quiescence that fires the response in the earlywood vessels, because the study area is located at low elevation, with relatively mild winter conditions; and the observed correlations were maximized in December and January. The existence of a response to winter conditions has been previously found for oaks growing in NW Iberia, not only for vessel elements (González-González et al. 2015), but also on tree-ring width (Rozas and García-González 2012a; Rozas and García-González 2012b; Rozas et al. 2009). However, a response to quiescence, and not to the dormant season, is more common for earlywood vessels (Fonti et al. 2007; González-González et al. 2014).

The physiological explanation for this relationship must be linked to the ring-porous anatomy of *Q. robur*. It is well-known that ring-porous trees form their first vessel elements before bud break (Pérez-de-Lis et al. 2015; Suzuki et al. 1996; Takahashi et al. 2013), and this row of vessels is fundamental for tree survival, because more than 90% of water is conducted by the earlywood of the most recent ring (Tyree and Cochard 1996). Consequently, they entirely rely on the reserves accumulated during the previous growing season to form at least the part of their vascular system and photosynthetic apparatus (El Zein et al. 2011; Gallé et al. 2007), so that carbohydrate storage is maximum before leaf fall in autumn and minimum just after leaf expansion (Barbaroux and Bréda 2002). Recent works indicate that carbohydrates can be limiting for tree growth, with the existence carbon depletion after stress episodes (Mitchell et al. 2013; Sevanto et al. 2014). Therefore, we expect that an excessive carbohydrate consumption due to a high respiration rate during winter will impact earlywood formation in spring.

But a high precipitation in the same period appears to be limiting for vessel size as well.

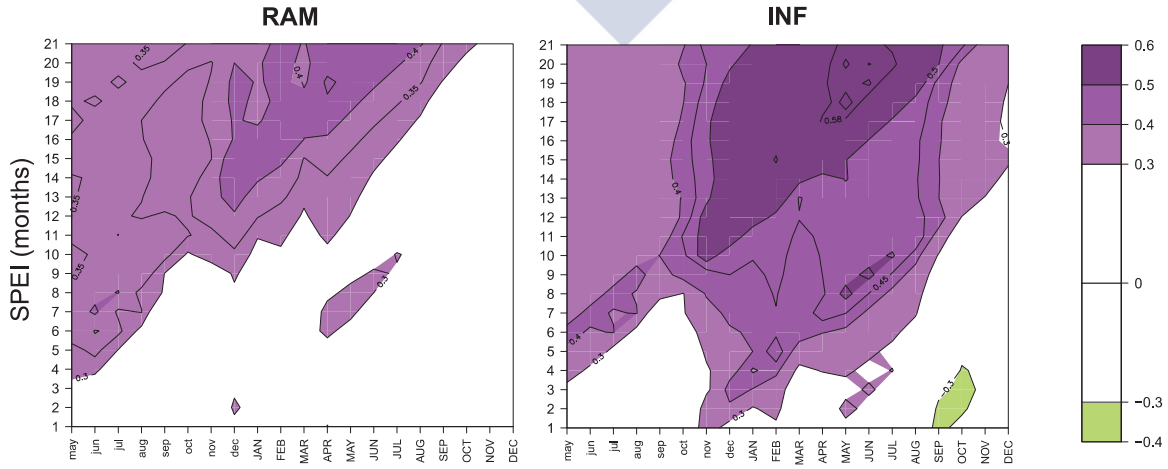

Fig. 7. Correlations between LW site chronologies and monthly SPEI values at different time scales (1-21) for the study period 1956-2013. Lower and uppercase letters correspond respectively to the months of the previous and current growth years.

This relationship cannot be interpreted as an association to temperature, because these variables are not positively correlated in winter, and also because the response was mostly observed only at the wettest site RAM. In our opinion, the effect of winter precipitation is due to waterlogging at this site, which produces some physiological reactions resulting in an additional consumption of reserves to overcome the dormant period. Soil water saturation produces to hypoxia, i.e, a low concentration of oxygen in the roots, which leads to adjustments in carbon metabolism (Kreuzwieser et al. 2004; Vartapetian and Jackson 1997; Visser et al. 2003), which involves an anaerobic pathway and the risk of self-poisoning by fermentation end-products (ethanol). Due to the low efficiency of this fermentative pathway, and the additional energy required to avoid cytoplasmic acidosis, the requirements to maintain the cellular energy status normally achieved through the oxidative respiration pathway can be as high as 19-fold under conditions of anoxia (Vartapetian and Jackson 1997). Therefore, carbohydrate consumption at RAM must be considerably increased because of its soil water regime.

These differences between sites turn to be more remarkable when observing the vessels formed outside the first row, as results were contrasting. At RAM, the effect of the dormant period also extended to the second and successive vessel rows, i.e., the effect of reserve fuel exerts a strong control on the whole earlywood. A reliable hypothesis for this behavior could be the existence of delay to achieve a positive balance of carbohydrates when photosynthesis is restablished, due to a low reserve availability at the moment of regrowth. On the contrary, vessels not located in the first row were nearly unrelated to the dormant season at INF, but mostly relied on spring temperature and precipitation instead, whereby a high temperature produces a faster differentiation resulting in smaller vessels (Pérez-de-Lis et al. 2015). The stronger limitation at the wet site RAM, extending for the whole period of earlywood formation, also explains the lowest agreement between sites for MVA-nr1, the more remarkable differences in vessel size

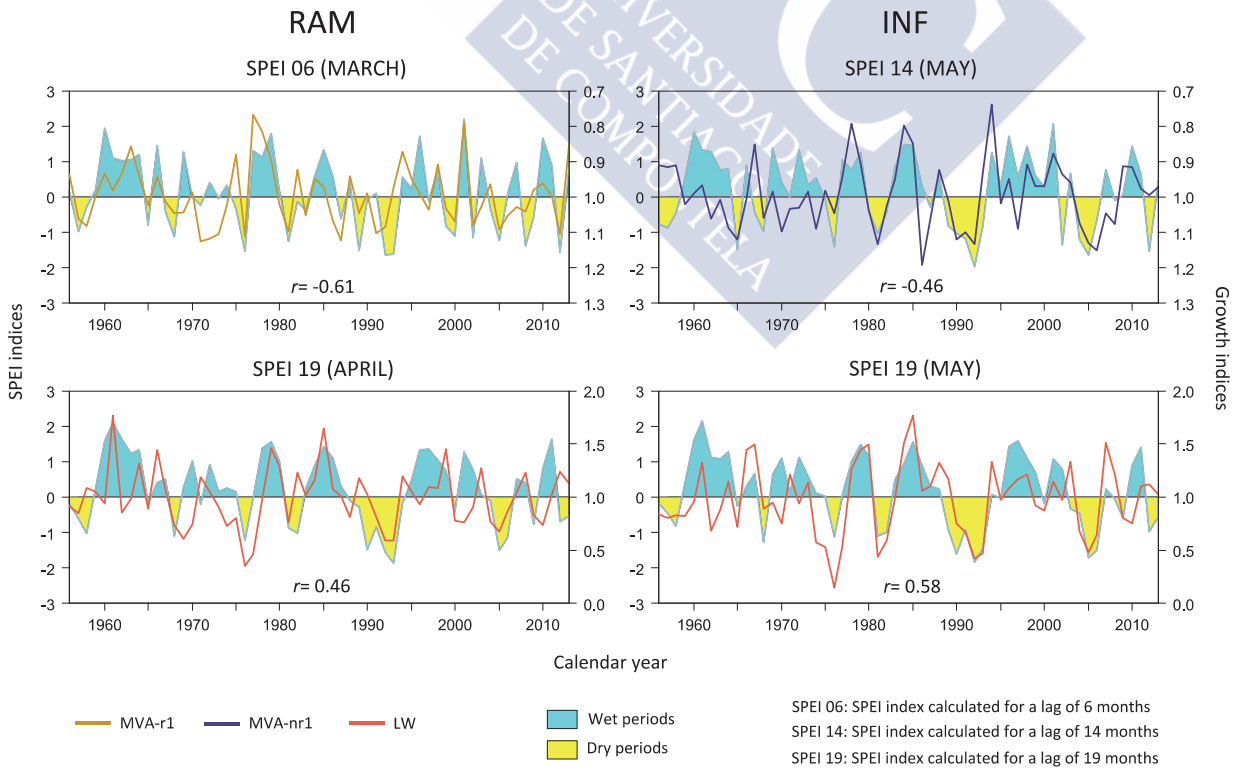

Fig. 8. Adjustment between growth variables and SPEI values for the months and time scales with the highest correlations at both stands, calculated for the study period 1956-2013.

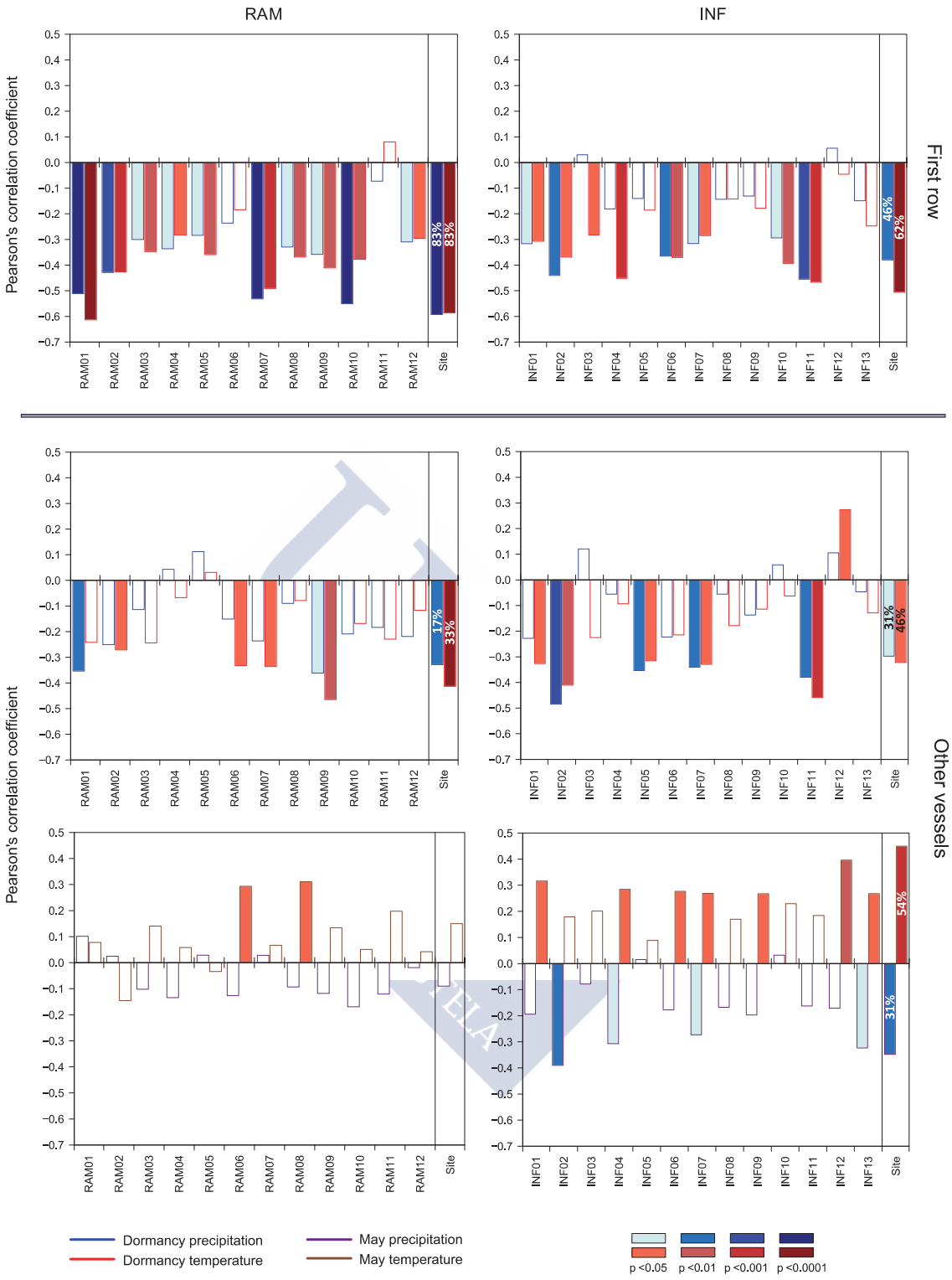

Fig. 9. Climate growth relationships for MVA of individual trees along the study period 1956-2013, expressed as bootstrapped correlations coeffient, adding the ratio of trees manifesting each site response. Climatic variables correspond to the temperature and precipitation for the dormant period (previous December to current February) and for May. Vessel rows are considered separately.

between rows for INF, and the high proportion of individual trees reacting at RAM. Furthermore, the use of the SPEI easily identified the differences between sites; there was a strong negative effect (rainy periods) of the dormant season at RAM, but MVA-r1; whereas the effect was much weaker, and occurred later in the season for MVA-nr1 at INF.

These results reinforce the evidence that conditions during the dormant period are very important for the radial growth of oaks at their distribution boundary in NW Iberia. Apart from the role of winter temperature evidenced by Rozas and García-González (2012a), several works point out the importance of winter precipitation. Thus, González-González et al. (2015) found that vessels of *Q. robur* were limited by water excess close to a river bank, radial increment of oaks were related to both temperature and precipitation in winter in an island under warm oceanic climate (Rozas et al. 2009), and Rozas and García-González (2012b) linked oak episodes of mortality to periods dominated by a high winter precipitation.

The former results confirmed one of our initial hypotheses, since temperature during dormancy was revealed as one of the limiting factors for cambial development. They also showed an additional effect, namely the effect of water excess, which appeared to be coupled to temperature regulating reserve consumption during the dormant period. However, our main hypothesis was based on the role of summer drought, i.e., the Mediterranean influence towards the distribution boundary of this oak species.

A classic dendrochronological analysis of climate-growth relationships through correlation functions did not show any clear effect of water availability during the growing season. Only INF had a certain positive response to precipitation during spring, whereas RAM probably lacked this response for not being limited due to the higher water holding capacity of the soil. In fact, winter recharge can be an important factor controlling growth at areas with Mediterranean climate, but following an Atlantic precipitation pattern in winter, as has been observed for *Q. ilex* in southern Portugal (Abrantes et al. 2013; Campelo et al. 2009). Apparently, the direct role of water availability on growth is not strong, but probably mediated through carbohydrate accumulation in the previous summer, and its consumption during the dormant and growth reactivation, which in turn affects latewood increment. Thus, water stress in the previous summer would limit photosynthesis, and therefore the accumulation of carbohydrates. This was evidenced by a negative response to temperature, which in turn indicates a high evapotranspirative demand for a species not adapted to avoid water loss, as opposed to sub-Mediterranean oaks (Sánchez-de-Dios et al. 2009). In addition, these results suggest that in boundary areas, i.e., under limiting conditions, oaks appear to prioritize reserve storage rather than growth during summer time, and consequently the absence of response in current summer would be due to an avoidance of this period for active radial growth, so that trees would guarantee enough reserves for dormancy and spring reactivation.

Although an effect of drought upon growth clearly exists at our sites, it was only the analysis of the SPEI that showed their influence, confirming its usefulness for forest ecosystems (Vicente-Serrano et al. 2014). Thus, the response to drought was not immediate, but extended for a longer period, starting from the previous growing season. Both sites had strong relationships for time scales of more than 20 months, especially for INF, and also lasted for a longer time

Conclusions

Our study was able to identify the main limitations for both earlywood and latewood growth of *Q. robur* in a region located near the limit of its natural distribution boundary in southern Europe. In view of our results, it is the balance of carbohydrates (i.e., synthesis, accumulation, and consumption) all throughout the season that controls wood formation, rather than a direct effect of climate at the moment of cambial activity.

We observed that winter conditions modulate the amount of reserves that will be available for earlywood formation. But the importance of the dormant season is a consequence of the limiting conditions imposed by the Mediterranean influence during the active period. As assimilation during the growing season is not high, trees appear to adapt a conservative strategy, and prioritize reserve storage rather than radial increment.

Since previous works in the region observed that mortality at the distribution boundaries of this species was produced after rainy winter periods (Rozas and García-González 2012b), the role of conditions during dormancy should be further explored as a stress factor for boundary oaks, including the analysis of wood formation and carbohydrate dynamics.

Acknowledgements

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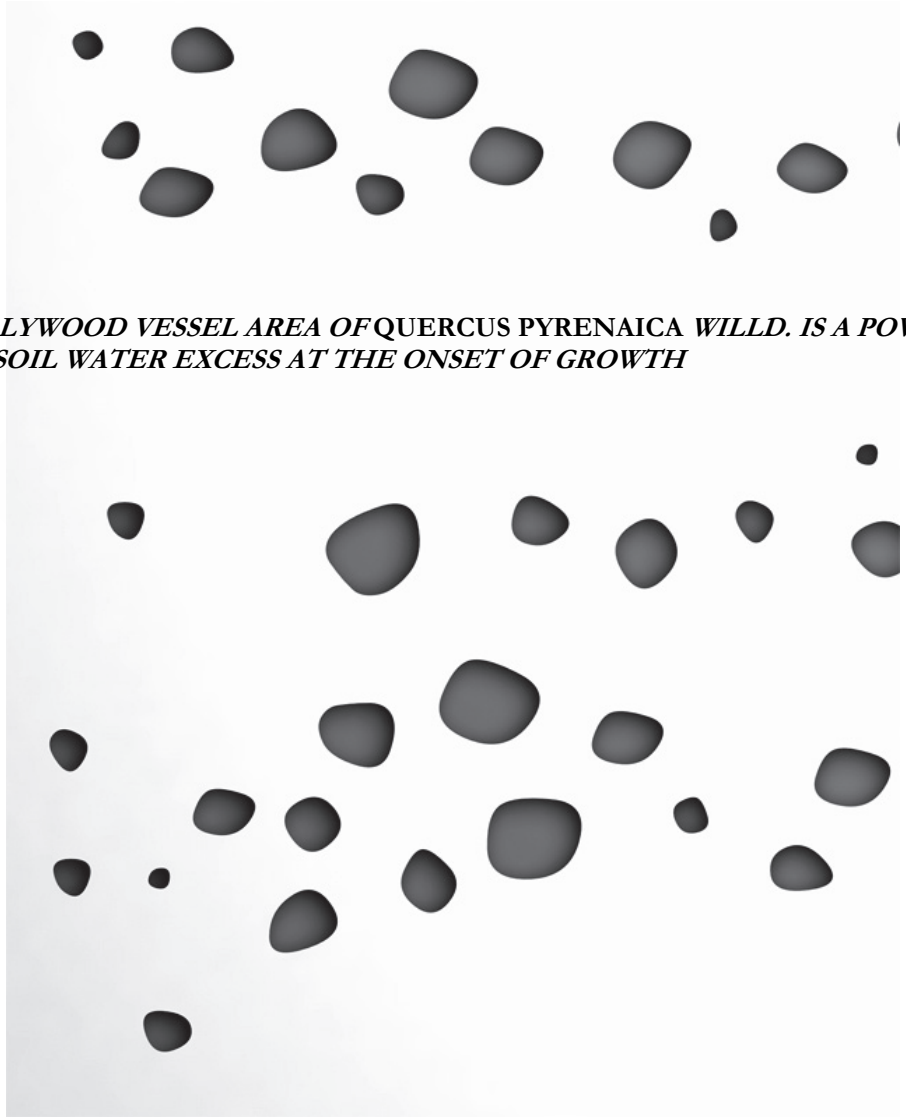

ARTICLE II. EARLYWOOD VESSEL AREA OF* QUERCUS PYRENAICA *WILLD. IS A POWERFUL INDICATOR OF SOIL WATER EXCESS AT THE ONSET OF GROWTH

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Abstract

We selected two sites dominated by the sub-Mediterranean oak *Quercus pyrenaica* Willd. close to its distribution boundary in the northwestern Iberian Peninsula, within a mountain region with a high winter precipitation. The sites differed in their soil water regime, corresponding to the edge of a peat bog, and to a moderate slope. We obtained tree-ring chronologies of total ring width (RW) and mean earlywood vessel area (MVA) for the period 1937-2002, and compared they response to climatic factors.

RW presented a much higher chronology quality than MVA, but was rather independent of climate, probably because of the presence of recurrent growth reductions resulting from past tree pollarding. In contrast, MVA was highly related to precipitation during April and May, whereby a high water availability was coupled to smaller vessels. However, we found remarkable differences between both sites, as trees growing on the peat soil responded later and with considerably lower intensity, as evidenced by correlations to local precipitation, common signal of MVA chronologies, and the number of trees recording the signal.

We hypothesize that waterlogging in early spring causes a delay in the response at the wettest site, which is recorded as soil desiccation begins; and also that the lowest variation within and between years accounts for the weaker signal. Climate-relationships at the driest site are mainly maintained by the vessels in the first row, whereas it is vessels expanded later in the season that show this relation for the moist site.

Our results confirm that vessel chronologies are reliable proxies of both regional and local climatic conditions, but only a careful optimization by the selection of vessel subsets does provide a complete view of their potential.

Keywords: Dendrochronology, tree ring, quantitative wood anatomy, climate proxy, soil water content

Introduction

Anatomical traits in tree rings have recently proven to be valuable proxy to study the relationships between tree growth and the environment, since they can be measured across sequences of tree rings (Fonti and García-González 2008; Gea-Izquierdo et al. 2012; González-González et al. 2014). The use of dendrochronological series of wood-anatomical features, which are a result of the plastic responses of trees to adapt to environmental constraints, has been proposed as a valuable tool to study global change (Fonti et al. 2010). Thus, this technique has been applied to investigate differences between species on the boundary of their distribution (González-González et al. 2014), to infer the behavior of a species along a gradient (Fonti and García-González 2008; Fonti et al. 2007), to study the main effect of climate on wood formation across an wide geographical area (Matisons and Brumelis 2012), or to understand the hydraulic adjustment of the xylem to face climate in the Mediterranean region (Abrantes et al. 2013; Gea-Izquierdo et al. 2012).

Several features have been used in the so-called quantitative wood anatomy, i.e., the measurement of anatomical features into annual time series. They usually involve the size of the conductive elements including both tracheids of conifers (Bryukhanova and Fonti 2013; Eilmann et al. 2006) or vessels of angiosperms (Fonti and García-González 2004; García-González and Eckstein 2003; Oladi et al. 2014). A few works were successful dealing with the vessels of diffuse-porous species, such as beech (Oladi et al. 2014; Sass and Eckstein 1995), poplar (Schume et al. 2004), or holm oak (Abrantes et al. 2013; Campelo et al. 2010), whereas the latewood of ring-porous trees has scarcely been analyzed (Woodcock 1989). But the most satisfactory results have been obtained with the large earlywood vessels of ring-porous trees (Fonti and García-González 2004). Thus, vessels of oak (Fonti and García-González 2008; González-González et al. 2014; Matisons

and Brumelis 2012) or chestnut (Fonti and García-González 2004; Fonti et al. 2007) provided very useful information about the role of climate on wood formation. In general, it was vessel size that recorded the best response, but other traits have also been considered in some cases (Gea-Izquierdo et al. 2012; González-González et al. 2015), because they proved to be also related to environmental factors. Both direct effect, i.e. those acting during the process of vessel expansion (García-González and Eckstein 2003), or indirect (Fonti et al. 2007; González-González et al. 2014) have been reported as reliable climate-growth relationships. Therefore, environmental constraints can affect not only vessel expansion, but also other processes in the tree such as reserve storage and consumption, or spring reactivation, resulting in responses that are found during dormancy and quiescence (Fonti et al. 2007; González-González et al. 2015), as well as during the previous growing season (González-González et al. 2014).

The sensitivity to climate found in the earlywood vessels of ring-porous trees is probably due to their peculiar characteristics as regards wood formation. Ring-porous trees usually form their first vessel elements at the beginning of the season before bud break (Suzuki et al. 1996; Takahashi et al. 2013), and therefore they must entirely rely on reserves previously accumulated (Barbaroux and Bréda 2002). Consequently, the formation of at least one row of earlywood vessels is essential for tree survival, because most of their conductive capacity is restricted to the last-formed ring (Ellmore and Ewers 1985). This is probably the reason that explains why the effect of climate is

commonly registered in these vessels, and therefore the signal obtained is of a great ecophysiological relevance. As a result, quantitative wood anatomy is in general applied to understand the role of climate variability on tree growth, and most recent works have been focused at identifying the effect of climate on a single site (García-González and Eckstein 2003), a region or gradient (Fonti and García-González 2008; Matisons and Brumelis 2012), or to try to infer the different behavior between sympatric species (González-González et al. 2014; González-González et al. 2015).

Oak species (*Quercus* spp.) are the dominant trees in many European deciduous forests and they have a ring-porous anatomy that makes them suitable for the construction of wood-anatomical time series (Fonti and García-González 2008; Fonti et al. 2010). Nemoral oaks (*Q. robur* L., *Q. petraea* (Matt.) Liebl.) extend from northern Europe until the boundary of the Mediterranean region, where they are progressively replaced by more drought-tolerant species. In the Iberian Peninsula, this role is undertaken by sub-Mediterranean, more drought-tolerant, marcescent oaks, among which *Q. pyrenaica* Willd. is probably the most important species (Sánchez-de-Dios et al. 2009). It mainly occurs in mountain areas within the western Iberian Peninsula, on acid soils, and in a subhumid to humid (occasionally hyperhumid) regime. The dendrochronological analysis of vessels of these sub-Mediterranean oaks appears to be a powerful method to understand their ecological behavior, and have been tested in several species such as *Q. pyrenaica* (González-González et al. 2014; González-González et al. 2015), *Q. faginea* Willd. (Corcuera et al. 2004), or *Q. canariensis* Willd. (Gea-Izquierdo et al. 2012). But the use of an indicator or 'proxy', such as the characteristics of the earlywood vessels, requires a detailed analysis of its sensitivity. Therefore, the comparison of these traits in trees growing under a same climatic regime but differing among a certain ecological characteristic is of great interest in order to evaluate their role as proxies.

In this paper, we aim to test whether differences in local factor within the same area are recorded in the variation of the earlywood vessels. For this, we deal with two populations of *Q. pyrenaica* that differ in their soil regime, namely in the duration of water saturation during dormancy. Trees grow under Atlantic climate close to the boundary between Atlantic and Mediterranean biogeographical regions. We hypothesize that a long period of prolonged waterlogging in winter and early spring influences the beginning of the active period at the wettest site, which in turn modifies the

characteristics of the earlywood vessels as compared to the driest site.

Materials and Methods

Study area and sites

The study sites were located in Northwestern Spain, within the Sanabria Lake Natural Park, which surrounds the largest glacier lake in the Iberian Peninsula (Fig. 1). This area lies within a granitic massif (42º05´N to 42º09´N and 6º38´W to 6º47´W), with a complex topography and peaks that reach over 2,000 m asl. Climate is Atlantic, but with a remarkable seasonality.

Winters are long and cold (until -11 ºC in January and February), with a frost period of at least seven months; and summers are short and hot (around 30 ºC in July and August), usually having a water deficit period (June-August). Due to this seasonality, the high rainfall (>1,000 mm/year) is mainly concentrated in autumn and winter, with precipitation as snow above 1,300 m asl. in winter.

From a biogeographical perspective, this area lies on the boundary between the Mediterranean and the Atlantic regions, but the study sites belong to first one (Rodríguez Guitián and Ramil Rego 2008). Natural woodlands selected correspond to the association *Holco molli-Quercetum pyrenaicae* (Rivas-Martínez 1987), and to EUNIS habitat type G1.7B112 (Sub-Atlantic humid Quercus pyrenaica forests) ((Davies et al. 2004), http://eunis.eea.europa.eu/habitats.jsp). They are relatively poor communities on acid soils, where *Q. pyrenaica* is accompanied by understory species like *Omphalodes nitida* (Willd.) Hoffmanns & Link, *Linaria triornithophora* (L.) Willd., *Lonicera periclymenum* L., *Physospermum cornubiense* (L.) DC., *Holcus mollis* L., or *Melampyrum pratense* L., i.e., many of them indicate a much more humid regime than usual for the Mediterranean region. We selected two woodlands dominated by *Quercus pyrenaica* (Table 1), which clearly differed as regards their topographic position.

Vegas de Galende (GAL) is a monospecific woodland considerably managed (firewood and sheepherding) in the past, with old and isolated trees. It was located at 1,000 m asl. at the bottom of a wide valley with glacial morphology, with hydro-perturbation processes over fluvio-glacial outwash deposits. The soil presented abundant organic matter, reaching the characteristics of a peat bog at certain locations. For this reason, many trees were scattered and isolated, or occurring only in edge areas where water excess was not extreme enough to exclude oak growth.

Monte Devesa, close to Ribadelago locality (RIB) was a deciduous woodland exclusively dominated by large *Q. pyrenaica* trees with no evidence of recent human activity. Smaller trees occured in the understory, including *Corylus avellana* L., *Ilex aquifolium* L., *Sorbus acuparia* L. or *Salix atrocinerea* Brot., especially towards the wettest areas of the forest. It was situated on a north-northeast moderate slope at 1,300 m asl., on a shallow soil with abundant granite fragments.

Sampling, ring width, and vessel measurements

We extracted at least two 5-mm increment cores at breast height from 28 (GAL) and 17 (RIB) dominant trees; the number of cores was higher at GAL, because several trees were hollow, and consequently not all extracted samples were usable. Cores were air-dried, mounted on wooden supports, and their surface cut and polished to obtain an optimal visualization of tree rings and vessel elements. A WSL sliding microtome (Gärtner and Nievergelt 2010) was used to obtain an even cross-sectional surface, but further manual polishing with progressively finer sandpaper (grain sizes from P220 to P1200, FEPA (Federation of European Producers of Abrasives) Abrasives) was necessary afterwards, due to the hardness of cores. We removed tyloses and wood dust inside the earlywood vessel lumina using high-pressure water blast (Fonti et al. 2002). Black printer ink was used on dry cores to dye the surfaces, thereafter earlywood vessels were filled with chalk dust (González-González et al. 2014); contrast achieved in final samples turned out optimal for image analysis.

We measured earlywood width (EW) and latewood width (LW) separately to the nearest

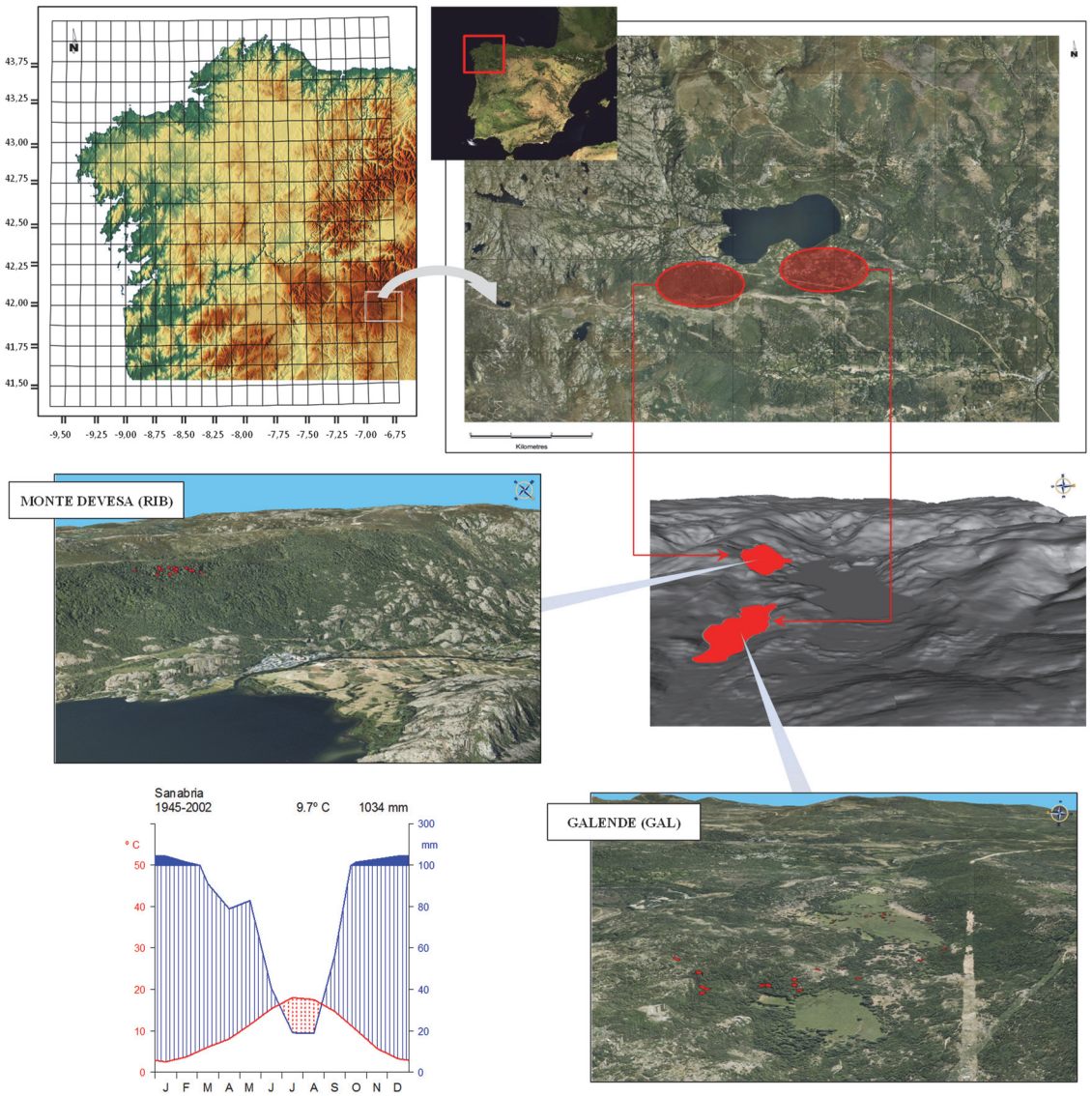

Fig.1. Location of the study area and topographic position of the sites and sampled trees. The climate diagram for Puebla de Sanabria weather station is shown.

0.001 mm, using a tree-ring measuring linear stage (Velmex TA UniSlide, Velmex Inc., Bloomfield NY, USA) combined with a binocular microscope (Olympus SZ60) at 20-40x magnification. The boundary between EW and LW was established based on vessel size and wood structure (García-González and Eckstein 2003), as earlywood vessels are much larger, and form up a continuous strip, whereas latewood vessels are smaller, and organize in flamelike groups along radial bands. Crossdating was performed on the series of total ring width (RW), calculated as the sum of EW and LW, by first comparing individual curves; the accuracy was statistically verified using COFECHA (Grissino-Mayer 2001).

The age of many studied trees spanned several centuries, but we restricted the anatomical analysis to the period 1937-2002. In order to carry out vessel measurements, we chose a subset of 10 trees per site, having no anomalies, breaks, or unsure dating, which were highly correlated to the local chronology. Sequences of digital images were captured directly from each selected core using a digital photo camera Nikon DMX 1200F, combined with a binocular optical stereomicroscope Nikon Eclipse 90i. Image resolution was 3600 × 2880 pixels. We stitched pictures belonging to the same core using PTGui Pro v.9.1.8 (New House Internet Services B.V., Rotterdam, The Netherlands), and the resulting images were saved into Tagged

Table 1. Physiographic characterization of the study sites.

Location	Code	Area (ha)	Elevation (m)	Facing	Slope (%)	Latitude (ºN)	Longitude (ºW)
Monte Devesa	**RIB**	80	1300	North	35-40	42.11	6.74
Vegas de Galende	**GAL**	112	1000	Southwest	< 5-15	42.11	6.69

Image File Format (TIFF) files to avoid loss of quality.

Earlywood vessels were automatically recognized and measured in ImageJ (Abramoff et al. 2004; Schneider et al. 2012) using the VesselJ plugin (García-González, not published). Image analysis based on an 8-bit (gray) threshold level, whereby vessel lumina (bright) were detected due to the contrast to the background tissue (dark). However, the presence of rays, dust spots, or other undesired objects also detected by the system hampered the correct recognition of all vessels. Hence, we applied several filters to the particles detected during the measurements in order to optimize the detection process. First, we fixed minimum vessel size at 10,000 μm^2 (Eilmann et al. 2006; Fonti and García-González 2004), and used circularity values to remove other objects that were mistaken for vessels. Despite all automatic procedures, manual corrections of the image were often needed to properly identify all conductive elements, as not all objects other than vessels could be excluded by these filters. We finally saved vessel outlines into files after, having optimized their shape by means of morphological operation (erode-dilate 2×2 one pass, and calculation of the convex hull).

Once all vessel lumina had been measured, we allocated their correct affiliation to the corresponding tree ring by Autovasos (García González, not published), and separated vessels belonging to first row (*r1*), which usually bear a higher sensitivity to climate (García-González and Fonti 2008), from those formed later in the season (*nr1*), in order to have subsets of vessels with a comparable ontogeny. We considered that the first row included all vessels located immediately after the ring boundary, and also those whose beginning did not exceed the imaginary line connecting the centers of the ring-boundary vessels. All data (ring widths and vessel measurements) from two processed increment cores were respectively averaged or pooled for each ring.

Variable survey and tree-ring analyses

From the vessel dataset obtained we calculated the mean area for all measured earlywood vessels (MVA) in each ring. Additionally, we obtained the same data for vessels belonging to first row, and for those not belonging to that row. In addition to MVA, we also included total ring width (RW) in the analyses (Table 2).

Growth-related trends were removed from all series by a cubic smoothing spline with 32-years stiffness and 50% VRF (Grissino-Mayer and Fritts 1997; Holmes et al. 1986). This spline stiffness is flexible enough to minimize strong trends in the ring-width series, and at the same time, using a same detrending method ensures that differences in the results between ring with and vessel size are not due to statistical procedures. Furthermore, the election of the detrending method should be negligible for the anatomical series, as trends were nearly abstent. After detrending, we obtained time series of growth indices by dividing each observed value by that estimated by the spline function (Fritts 1976), and the indices were averaged into a chronology for each variable and site, using a biweight robust mean to minimize the effects outliers (Mosteller and Tukey 1977).

Time period used for analyses was 1937-2002. Individual series and chronologies were characterized by standard descriptive statistics, as the mean sensitivity (*MS*) and the first order autocorrelation coefficient (*AR1*). *MS* is an indicator to interannual variation (Douglas 1935), and *AR1* shows the influence of previous year growth over a particular year. The statistical quality of each single chronology was evaluated with several coefficients commonly

used in dendrochronology (Cook et al. 1992) such as the mean correlation between trees (*Rbt*), the variance in the first eigenvector *(%Var*), the signal to noise ratio (*SNR*), and the expressed population signal (*EPS*). *Rbt* is the mean value of all possible Pearson´s cross-correlation coefficients, *%Var* originates from a principal component analysis on all individual series, *SNR* is the resulting value from the division between *Rbt* and the statistical amount relative to noise, and *EPS* indicates the extent to which the simple size is representative of a theorical population with an infinite number of individuals (Wigley et al. 1984).

We evaluated the agreement in the information provided by the same variable at both study sites, and between MVA and RW at each site, by means of Pearson's cross-correlation coefficient (*r*). We also studied the ordination of individual trees for both MVA and RW, in order to establish the importance of the differences between sites. This was accomplished by principal component analysis (PCA), applied on the correlation matrix of all selected trees of both plots, and using varimax rotation for the extraction of the two first principal components.

Climate-growth relationships

Climatic data used to calculate relationships to climate were obtained from both local and gridded records. We employed regional precipitation series for 1945-2002 from Puebla de Sanabria meteorological station (42º 03' N, 6º 38' W, 960 m asl.), and also estimated data from CRU TS 3.21 dataset (series of monthly mean, and average daily maximum and minimum temperatures, for 1901-2012) taken from the Climate Explorer of the Royal Netherlands Meteorological Institute (http://climexp.knmi.nl/) for the 0.5º× 0.5º grid in which the study area is located. We observed that estimated CRU precipitation series were less accurate than the local meteorological station. On the contrary, local temperature records had a very good agreement

to gridded data, but they were incomplete, and accounted only for mean monthly temperature. Therefore, both data sources were necessary to establish climate-growth relationships.

We considered the period 1945-2002 (58 years) for the establishment of climate-growth relationships, because it was covered by a complete climatic data record. The analyses were performed by means of correlation functions, i.e., Pearson's correlation coefficients between monthly meteorological data and the indexed chronologies of ring width and earlywood variables, calculated all throughout the period that could influence wood formation; in this case, it comprised from previous May to current June (and to October for RW). Significance level of the correlation coefficients was determined using the bootstrap technique (Guiot 1991); we computed 10,000 iterations for each coefficient, and applied the correction proposed by Mason and Mimmack (1992) for the percentile bootstrap confidence intervals, as Pearson's correlation coefficients are not always normally distributed. Correlation functions were computed not only for the chronologies, but also on single trees, because individual responses are often capable of providing valuable additional information (Rozas and Olano 2013). García-González and Fonti (2006) recommended that vessels should be progressively selected to maximize the climatic signal, since cells formed at different times can contribute to a more accurate knowledge of the allocation of this signal throughout the ring. In fact, not all vessels bear the same information, nor with the same intensity (Fonti et al. 2010). Although we initially considered vessels formed in the first row and those formed later separately, which is a common practice to improve the signal contained (González-González et al. 2014; Hroš and Vavrčík 2014), this procedure hardly added additional information. Therefore, we applied a progressive filter to retain or remove vessels at each step, as recommended by (García-González and Fonti 2006), applying the percentile method. The filters were performed in two directions, i.e., we remove the smallest vessels, retaining the signal contained in the

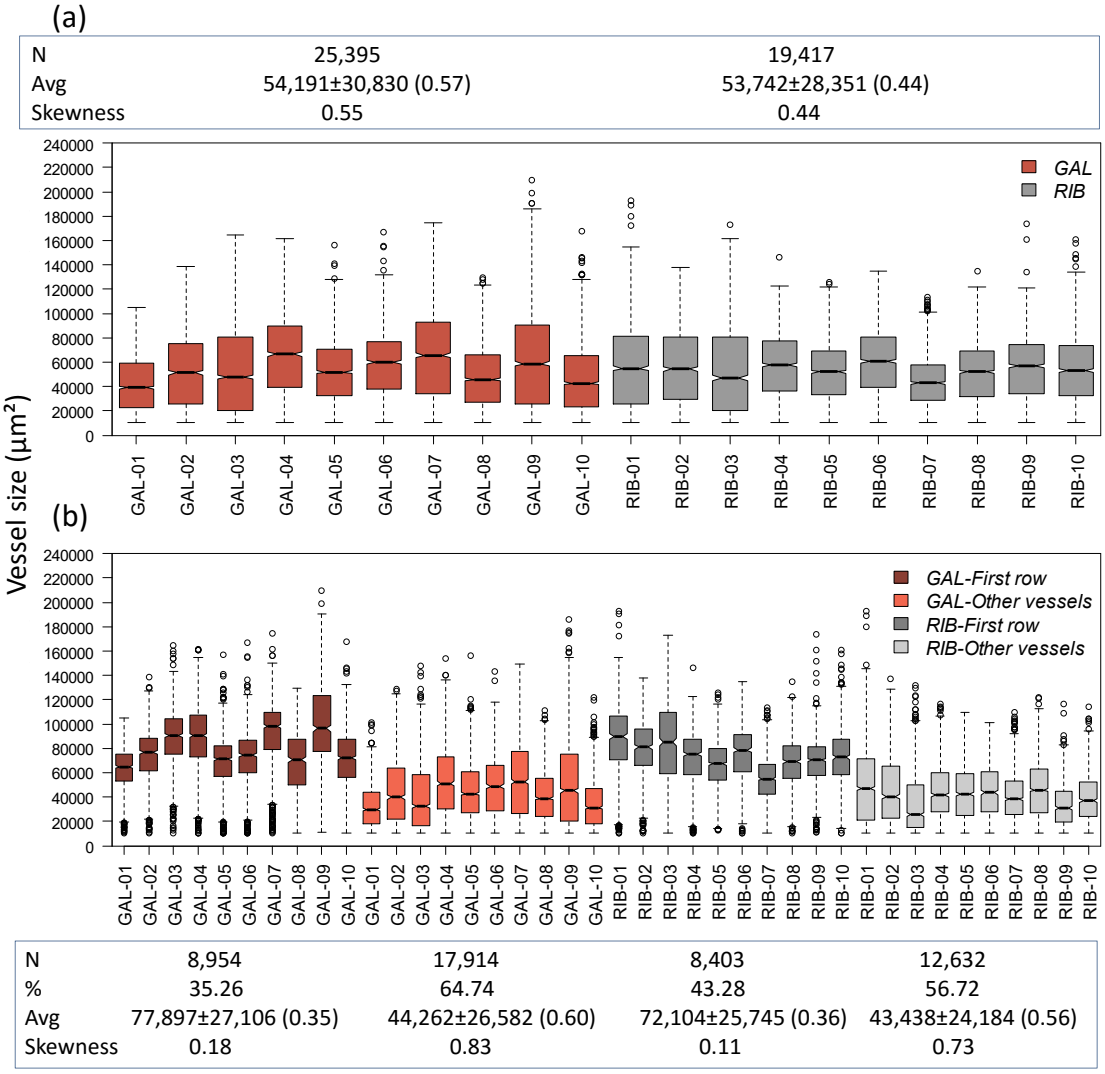

Fig. 2. Boxplots of vessel distributions and descriptive statistics of Galende (GAL) and Monte Devesa (RIB), for all earlywood vessels (a) and split on first row, and other vessels (b), along the study period 1937-2002.

largest ones; and a second filter removed the largest vessels instead. For this, we used the distribution of vessel size within each ring, and we removed at each step 1% of the largest or smallest vessels respectively. After a percentile had been removed, we recalculated individual series of MVA on the *n*-percent of the vessels that had been retained, the corresponding mean chronologies, and the subsequent analysis of climate-growth relationships. In order to proceed with these computations, we developed a programming routine using Delphi XE7 (Embarcadero Technologies, San Francisco, CA, USA).

Results

Vessel size distribution and chronology assessment

We measured more vessels at GAL (25,395) than at RIB (19,417), but both sites showed a similar earlywood vessel size (54,109 µm^2 vs. 53,742 µm^2). Distributions were clearly skewed, being small vessels much more frequent (Fig. 2a), and values for all statistics were not remarkably different between sites. The study of vessel distribution separated by row provided more information about size distribution (Fig. 2b). The first row contained proportionally less vessels at GAL (35.3%) than at RIB (43.3%) and these, although still a little skewed, tended

to be normally distributed at both sites, whereas small vessels were clearly predominant outside this row. First-row vessels were considerably larger than the others, and also slightly larger at GAL than at RIB, whereas vessels not located in this row did not differ between sites.

In general, principal component analysis clearly separated both sites on the space defined by the two first eigenvectors (Fig. 3), with only 1-2 trees that did not group with the whole site. Such discrimination was clear for both MVA and RW, confirming notable differences between sites.

MVA and RW chronologies (Fig. 4) were completely unrelated at GAL (r=–0.16, n.s.), and though significant, not strongly correlated at RIB (r=–0.44, P<0.01). MVA chronologies were not autocorrelated, and had a very low year-to-year variability (*MS* below 0.10), but yet some variation that was able to be analyzed dendrochronologically. Common signal was also low as compared to usual values in tree-ring series, but not within the expected range for earlywood vessel variables. In fact, *Rbt* for RIB can be considered higher than the rule for MVA, being also much higher than at GAL, which evidenced a common behavior of most trees at this site.

RW had a much stronger common signal than MVA for all parameters of the common interval, and can be considered optimal for further dendrochronological analysis. Year-to-year variability was however not high (0.12-0.15), and the series were still autocorrelated; both *MS* and *AR1* were higher for GAL. Contrarily to MVA, GAL showed the best statistics for RW chronologies. As compared to MVA, there was a big impact of growth reductions on the series, especially at GAL, which were completely abscent when analyzing earlywood, confirming again that both variables account for a different kind of information.

The comparison between both site chronologies also indicated the different behavior of earlywood and latewood. GAL and RIB were highly correlated when analyzing RW (r=0.60; P<10^{-5}), whereas the correlation coefficient was rather low for MVA (r=0.31; P<0.05), indicating that there is much more variability within earlywood that can be studied in order to understand the different climate-growth relationships of both stands.

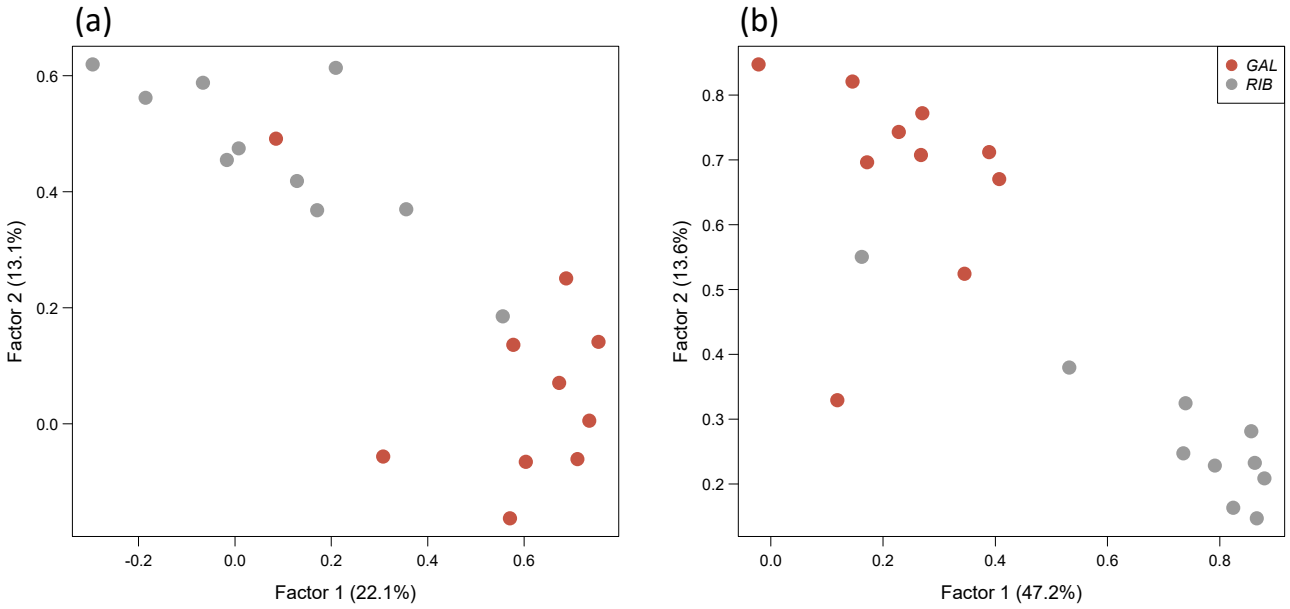

Fig. 3. Principal component analysis showing the ordination of all trees for (a) mean vessel area (MVA), and (b) total ring width (RW), along the study period 1937-2002.[i]

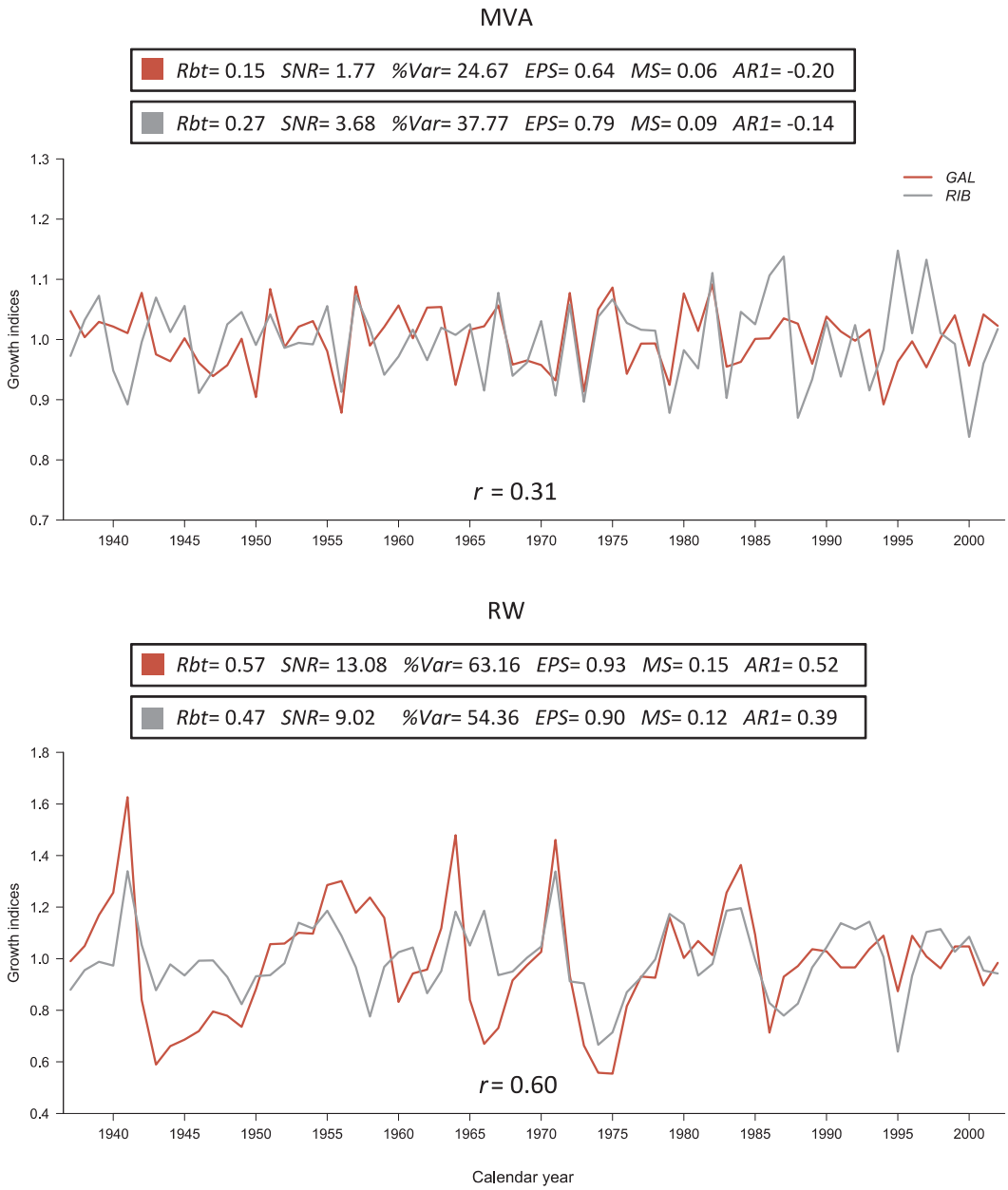

Fig.4. Comparison between site chronologies of mean vessel area (MVA) and total ring width (RW) for the study period 1937-2002, covered by at least 10 trees; statistics of chronology quality assessment are shown. *Rbt*: mean correlation between trees; *SNR*: signal-to-noise ratio; *%Var*: variance retained by the first eigenvector; *EPS*: expressed population signal; *MS*: mean sensitivity; *AR1*: first order autocorrelation coefficient.

Climate growth-relationships and optimization of climatic signal

Although common signal of MVA chronologies was very low as compared to RW, their relationships to climate were strong and reliable (Fig. 5). Both sites were negatively correlated to precipitation, but at different moments of the active growing season. While GAL was highly related to May precipitation (r=–0.47, P<0.0001), RIB responded earlier in April, and the relation was stronger (r=–0.58, P<0.0001); in addition, there was also a remarkable relation to temperature in the same month. Despite GAL was also correlated to precipitation in April, and RIB to May (r=–0.29, P<0.05; r=–

0.27, $P<0.05$) respectively), correlations were much lower than those above-mentioned, and certainly related to some vessels that form earlier or later than the majority. Other relationships found, namely low correlations to temperature in the previous season at GAL, are of less interest, but confirm the fact that the role of spring precipitation is less important at GAL than at RIB.

Climate-relationships to RW (graph not shown) were generally very weak, and linked to April rainfall at both stands, but stronger at RIB (r= 0.46, $P<0.001$), than at GAL (r=0.31, $P<0.05$). A certain effect of other climatic variables was also recorded, but correlations were low, never reaching $P<0.01$; this included precipitation during last November at both sites (r= 0.28 at GAL, r= 0.32 at RIB), July at GAL (r= 0.33), and September at RIB (r= –0.27), and also temperature responses at RIB for the former two months (r= 0.31 and r= 0.26, respectively).

The importance of the relationship to spring precipitation can be seen when analyzing individual relationships of trees in April and May (Fig. 6). Whereas only four out of the ten trees had a significant correlation to May precipitation at GAL, nine out of ten trees were correlated to April at RIB, and the significance level of the correlations was considerably higher ($P<0.05$ to $P<0.0001$). This result is coherent with the values of common signal obtained for MVA. Furthermore, this individual analysis clearly showed the shifted response at GAL, whereby most trees at RIB were only correlated to April precipitation, and so did GAL to May.

But the differences in the moment and intensity in the relationship of MVA to climate are better understood when analyzing different subsets of vessels. Though weakly, the study of rows separately pointed out that the climatic signal was collected by a different group of vessels at each site (Table 2). In fact, the response at RIB was almost exclusively contained in the first row, while the signal at GAL was completely lost when splitting the ring, i.e., the climatic information seems to be distributed all along the earlywood or at the transition between the first row and the rest. For this reason, the analysis of separated rows was of limited value

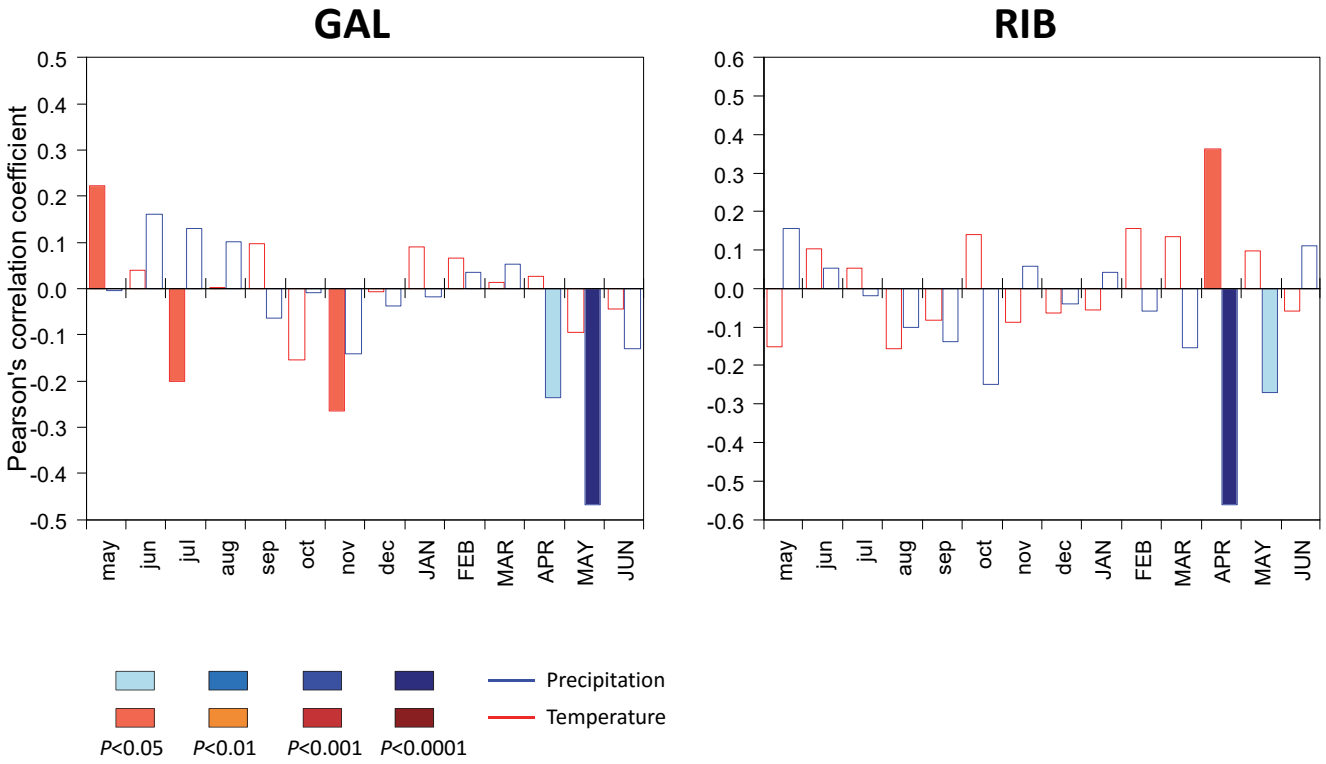

Fig. 5. Climate growth-relationships for mean vessel area (MVA), expressed as bootstrapped correlation coefficient, along the study period 1937-2002. Lower and uppercase letters correspond respectively to the months of the previous and current growth years.

to establish relationships to climate, and the best optimization was provided by the size filters (Fig. 7).

Progressively removing one percentile of the smallest or largest vessels within each ring provided notable differences between sites, and help clarifying what vessels were recording the signal more efficiently. At GAL, the removal of small vessels lowered the correlation to May precipitation, even from the earliest computational stages, whereas the reduction of signal was much more gentle if large vessels were eliminated. On the contrary, correlation between MVA and April precipitation at RIB decreased fast from the beginning if large vessels were removed, whereas it was necessary to exclude ca. 90% of the smallest vessels to be able to see any impact on the results. Therefore, the climatic signal at RIB was contained in the largest vessels, usually formed at the beginning of the ring, but that was not the case for GAL.

Discussion

Quality and climatic signal of chronologies

RW had a stronger common signal and a higher year-to-year variability than MVA, apparently showing that it is a more appropriate variable for dendrochronological analyses. Previous works reported similar values for RW of oaks in northern Spain (González-González et al. 2014; Rozas 2005; Rozas et al. 2009), or even lower that the ones found at our study sites. On the other hand, the low common signal and year-to-year variability of MVA chronologies appears to be the rule not only for most ring-porous species such as *Quercus* spp. (Fonti and García-González 2008; García-González and Eckstein 2003; Gea-Izquierdo et al. 2012; González-González et al. 2014) or *Castanea sativa (Fonti and García-González 2004)*, but also for diffuse-porous species such as the evergreen *Quercus ilex* (Campelo et al. 2010). However, these parameters only indicate statistical quality and not the recorded climatic information (Campelo et al. 2010), which can be optimal despite a low common signal as is usually found in ring-porous trees (Fonti et al. 2010). For this reason, it is necessary to compare chronologies to

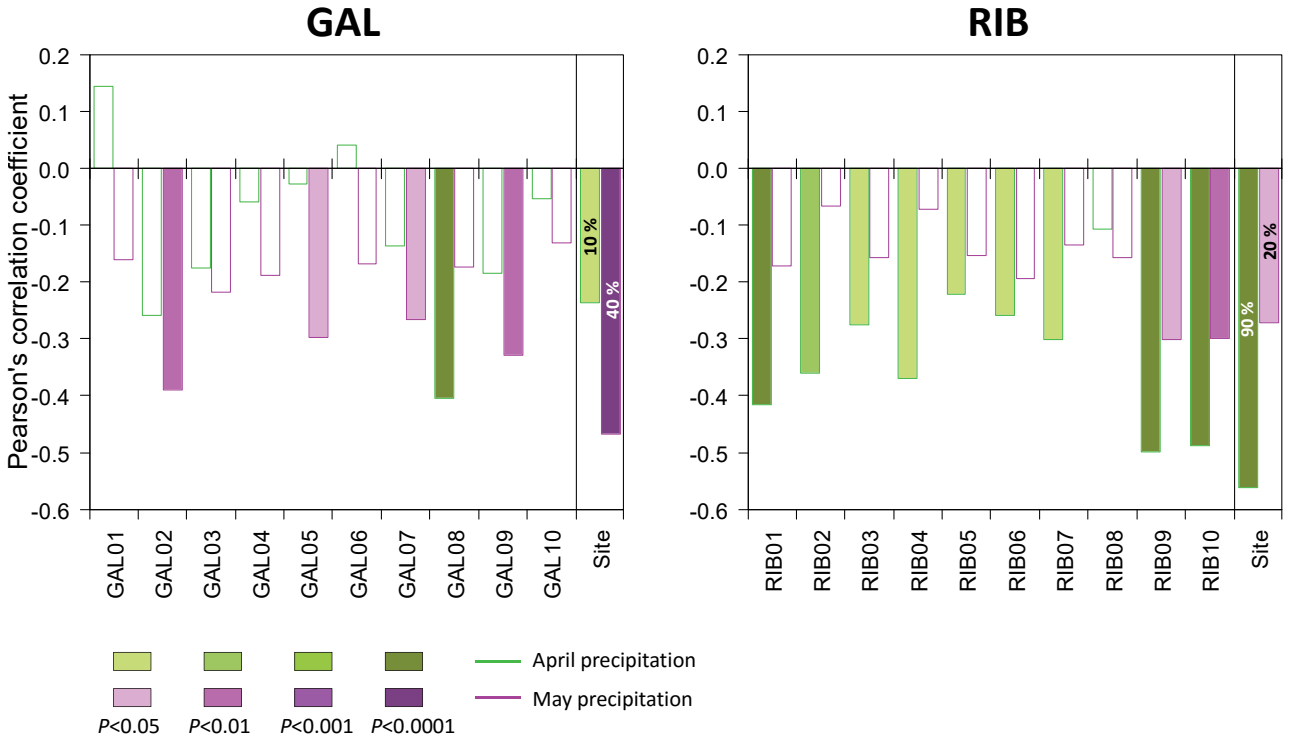

Fig. 6. Correlations between mean vessel area (MVA) of single trees and the main climate variables related (precipitation in April and May), along the study period 1937-2002.

climatic records in order to evaluate its potential to understand the effect of climate on growth.

Our results showed that MVA was close related to climate, and this was not the case for RW. In fact, MVA presented a high correlation to spring conditions, i.e, the time of vessel formation, indicating that this process is probably strongly controlled by climate. However, we did not find any reliable relationship between RW and the meteorological records. This appears to be a common situation when analyzing climate-growth relations in oaks, as has been previously observed in other works dealing with both anatomical and ring-width measurements (Fonti et al. 2007; González-González et al. 2014; González-González et al. 2015), especially under the lack of a prevalent limiting factor. In view of this, Fonti and García-González (2008) proposed that earlywood vessel size is a powerful potential proxy in mesic areas, where climatic conditions are apparently not limiting, because it showed strong correlations to climate where RW did not. Furthermore, our results also point out that earlywood and latewood are controlled by different physiological processes, and do not contain similar ecological information (Fonti and García-González 2004).

The understanding of the main limiting factors for earlywood growth is not straightforward, because it requires a detailed knowledge of wood formation dynamics. The only studies involving *Q. pyrenaica* are scarce and recent (González-González et al. 2013; Pérez-de-Lis et al. 2015), whereas dendroecological analysis of the earlywood vessels showed different results depending on the site of geographical location (González-González et al. 2014; González-González et al. 2015). But radial increment of (sub)-Mediterranean oaks is mostly controlled by water availability (Campelo et al. 2009; Gea-Izquierdo and Cañellas 2013; Gea-Izquierdo et al. 2012), which is apparently not limiting at the study sites. *Q. pyrenaica* tolerates summer drought, but mainly occurs in subhumid Mediterranean mountains (Sánchez-de-Dios et al. 2009), so that moisture regime of the study area should not be an issue for the species; in fact, annual precipitation is higher than 1,000 mm/yr, with an important water recharging

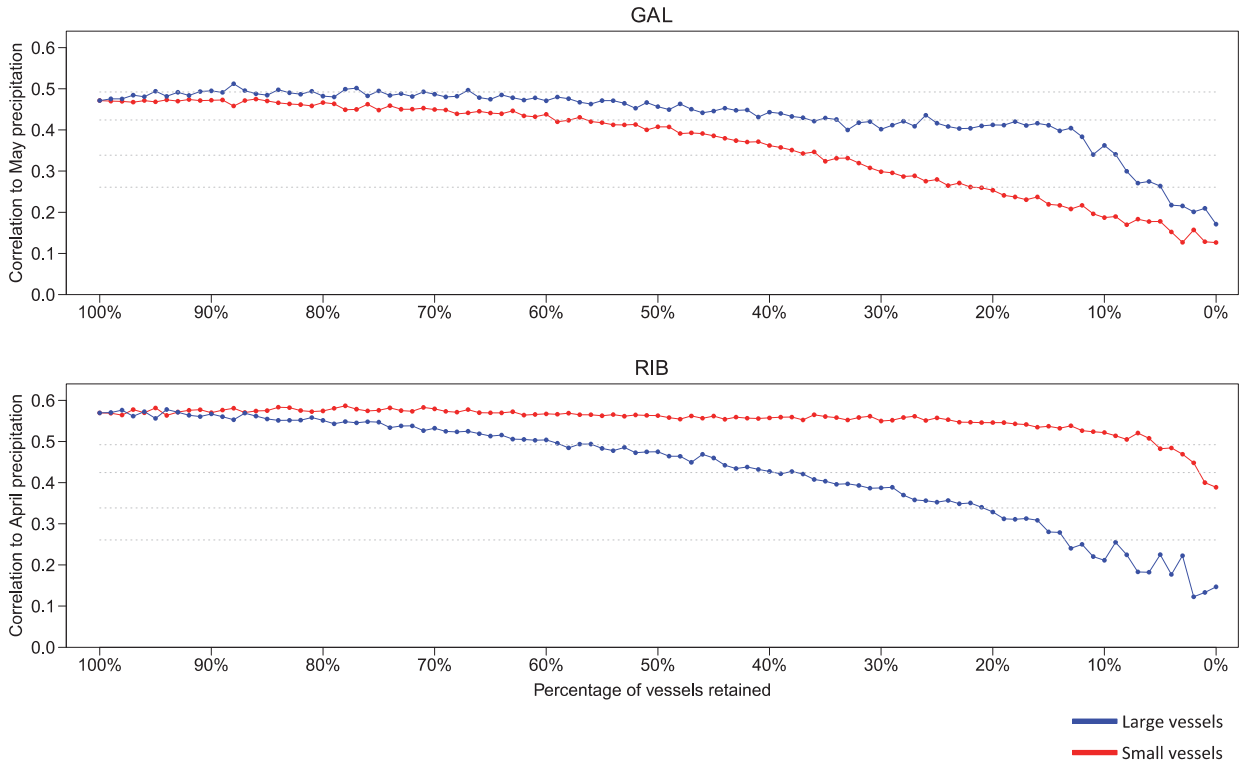

Fig. 7. Progressive removal of vessels, filtering out 1% of the vessels per ring at each step. Correlation is shown for the climatic variables most related to MVA (May precipitation at GAL, April precipitation at RIB).

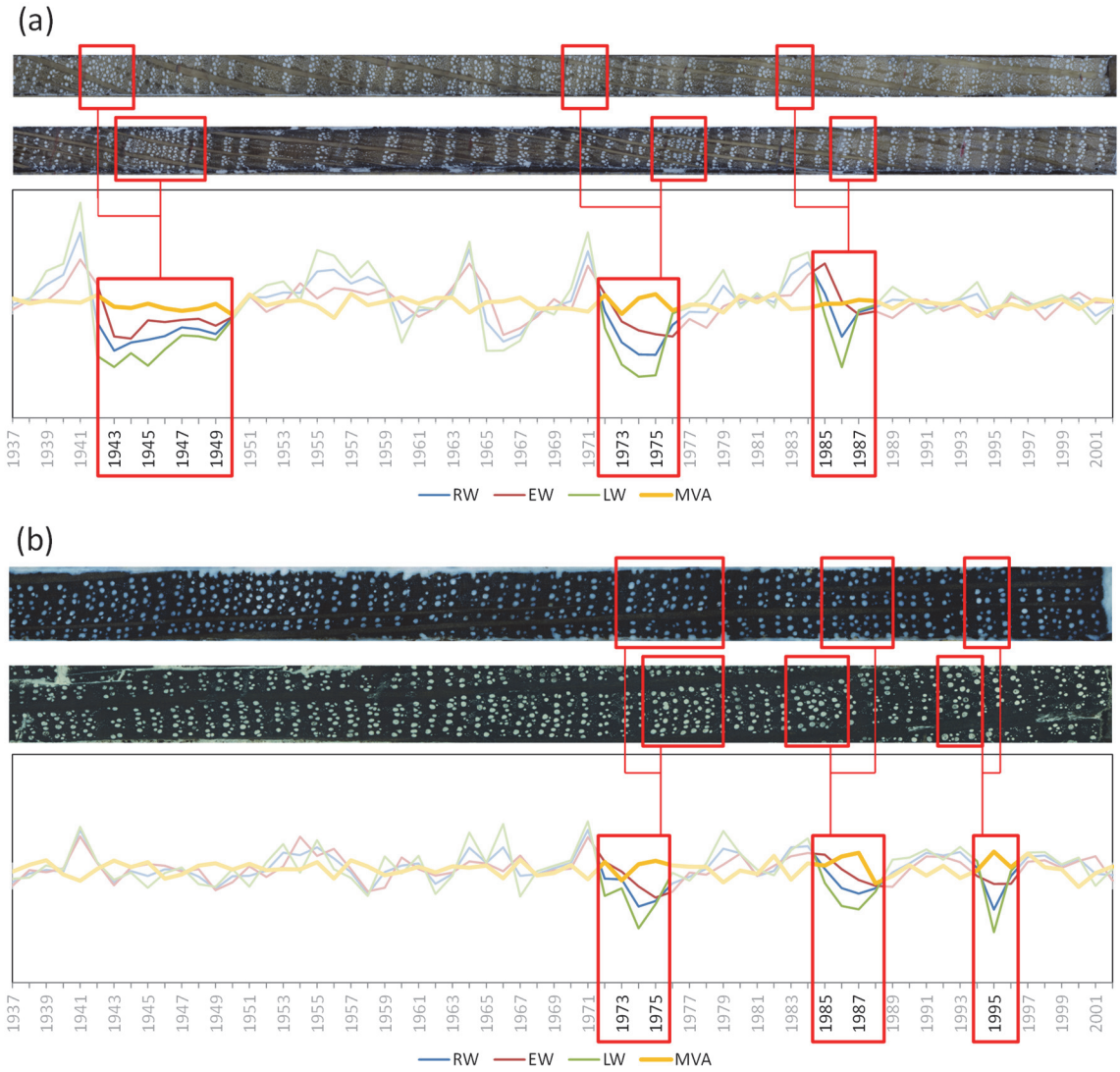

Fig. 8. Chronologies of the study variables, showing the appearance of the tree rings during the main episodes of growth reductions, not recorded by MVA, observed in most sampled trees at GAL (a) and RIB (b). *RW*: total ring width; *EW*: earlywood width; *LW*: latewood width; *MVA*: mean vessel area.

during winter. Thus, conditions are probably close to optimal for the species, at least during summer time.

The lack of climatic response of RW is probably not due to favorable conditions, but because of disturbances that considerably affect radial increment. We observed recurrent episodes of severe growth reduction during the study period, probably related to biotic factors (e.g. insect outbreaks) or human activity (pollarding), which must be masking the effect of climate. However, the effect of these reductions mainly affected latewood width (and consequently RW), it was smoothed in earlywood width, and had no effect on MVA, i.e., vessel chronologies did not register these periods at all (Fig. 8). This explains the lower sensitivity and the lack of autocorrelation of MVA, and especially the strong agreement found in climate-growth relationships. In turn, this explains why MVA appears to be a more promising climatic proxy.

Ecological meaning and relevance of climate-growth relationships

We found a strong negative relationship between vessel size and precipitation. The linkage between both variables has often been observed for ring-porous species not only in spring, but at different moments of the season, namely the previous year (González-González

et al. 2014; Matisons and Brumelis 2012), or dormant period (González-González et al. 2015). Furthermore, correlations between MVA and precipitation during the period of vessel formation can be positive (García-González and Eckstein 2003) or negative (Fonti and García-González 2008; García-González and Eckstein 2003), showing that the response to temperature may be cause by many different mechanisms.

In our case, there were remarkable differences between both sites, despite being close to each other. This was appreciated in the low correlation between MVA chronologies, the clear separation of individual trees, and in the differences in timing and intensity of climate-growth relationships. The reason for this must be the contrasting site conditions, trees at GAL grew on a peat bog, and therefore the soil should be saturated of water after the rainy period in winter, whereas RIB was located on a slope, so that the soil was rocky and sandier and had a clear run-off.

We hypothesize that the period of waterlogging at the wettest site GAL results in a retardation of growth in early spring in relation to RIB, which in turn causes a delayed and weaker response to environmental conditions. Unfortunately, there is nearly no information about the impact of flooding on deciduous trees at the end of dormancy (Wang et al. 2015), although the overall effects on active growth are well documented (Alaoui-Sossé et al. 2005; Gérard et al. 2008; Parelle et al. 2006; Vartapetian and Jackson 1997). But according to Wang et al. (2015), responses to winter waterlogging may differ between deciduous and perennial species, because the initiation of photosynthesis requires leaf growth.

One of the main effects of soil water surplus is oxygen deprivation, which leads to fermentative metabolism pathways, the accumulation of self-poisoning ethanol, and consequently a considerably increased consumption of carbohydrates (Vartapetian and Jackson 1997). Other process affected also involve photosynthesis and nutrient concentrations (Dreyer 1994; Parelle et al. 2006), root water uptake (Aroca et al. 2012), and at the long term, anatomical adaptations (Wang et al. 2015). Thus, water excess during the dormant period has been found to affect both ring width (Rozas and García-González 2012) and earlywood vessels (González-González et al. 2015), which has been related to excessive consumption of reserves in both cases. However, we do not think that carbon limitation during dormancy is the driving force behind our results, since the relationships to MVA are considerably stronger in the absence of waterlogging, and are unrelated to the dormant season.

The time and duration of the response of earlywood vessels to climate suggests that there is probably a fairly direct relationship between precipitation and vessel size, i.e., the effect of water availability is operating at the moment of vessel enlargement. According to Pérez-de-Lis et al. (2015), the duration of the phase of expanding vessels can span up to nearly two months for this species; this is consistent with the duration of the responses observed, which extended for April and May. During this period, a high water content in the soil results in smaller vessels, though the physiological mechanism has not been elucidated, and is out of the scope of this paper. Leaf unfolding occurs around late May at this mountain study area, whereby cambial divisions have been observed ca. 5-6 weeks before bud break for *Q. pyrenaica* in lower areas of NW Iberia (Pérez-de-Lis et al. 2015).

The difference in the timing and intensity of the relationship to spring precipitation is the result of the contrasting soil water regime. The position on a hillslope at RIB prevents soil from being saturated at the moment of cambial reactivation, and therefore the effect of precipitation is clear for the beginning, keeping the soil saturated in rainy periods, but drier in the absence of rainfall. As a result, trees responded to variations in soil water content at the beginning of the season (April), and consequently this response was mainly registered by the vessels in the first row; the much lower correlation to precipitation in May was probably recorded by the most recent vessels still responding to spring rainfall.

This also explains why the removal of small vessels (mostly formed later in the season) did

not impact the relation to April precipitation, but the largest ones were required to optimize the signal. In addition, there was a positive correlation to temperature in the same period, apparently not coupled to rainfall, which is probably indicating the minimum threshold to initiate cambial activity, and has already been pointed out by other authors for the same species under mountain conditions (Gea-Izquierdo and Cañellas 2014). The consistency in the signal of April precipitation was also evidenced by the significant correlation found for MVA in 90% of the study trees, and also by the highest common signal of MVA at this site.

As regards the waterlogged site GAL, the response to spring precipitation occurred later, despite being at a lower elevation (ca. 300 m less). Correlation to April was low, but it was May that maximized the relationship to climate. According to our hypothesis, soil was probably saturated in water during most April, and therefore conditions for vessel differentiation were similar for most years. Whether waterlogging can affect phenology when breaking dormancy is not clear (Wang et al. 2015), but a delay, slow-down of processes, or the above-mentioned lack of year-to-year variation, are all compatible with our results. In fact, (Pérez-de-Lis et al. 2015) found that the duration of vessel enlargement barely influenced earlywood vessel size in *Q. pyrenaica*, so that expansion rates should be involved.

Trees at GAL appear to be related to spring precipitation as soon as soil desiccation around the peat bog can begin. The lower correlation and the reduced amount of trees responding is another evidence of the less variation in soil water content, which also imposes more uniform conditions throughout the years. Further results point out that the signal are not recorded by the first earlywood vessels, but probably those formed a few weeks later, namely from the second half of April. Considering vessel rows separately led to a complete loss of climate-growth relationships, and therefore it is plausible that first-row vessels more separated from the tree-ring boundary, together with the first vessels formed outside the first row, are the ones expanding during the moment the precipitation signal is recorded. The similar trend when filtering out small and large vessels, and the loss of signal with a low amount of small vessel retained, also supports the idea that it is vessels in 'intermediate' position that optimize the signal. Finally, the lack of response to temperature, as oppose to RIB, is probably an evidence that the response of the trees occurs later, when temperature thresholds are no more limiting. Furthermore, climatic signal is recorded during a more prolonged time span, because the expansion of vessels involves more rows, and also heterogeneity because of the different position within the site (more or less prone to soil desiccation) may favor individual expressions in detriment of a common response to climate (Rozas and Olano 2013).

Concluding remarks

Using the comparative analysis of chronologies of tree-ring width and earlywood vessel size for *Q. pyrenaica* at two contrasting sites we were able to establish that i) the earlywood vessels contain a strong climatic signal, and ii) this signal is a powerful indicator of local ecological conditions. In contrast, ring width resulted to be much more limiting in order to gain climatic information, probably for being much more influenced by other events as forest disturbances. Overall, this confirms the validity of using the earlywood vessels of ring-porous oaks as reliable proxies in areas where climatic conditions are not particularly limiting for tree growth (Fonti and García-González 2008; Fonti et al. 2010; González-González et al. 2014).

Specifically, we were able to demonstrate that the earlywood vessel size of *Q. pyrenaica* under a strong Atlantic influence in a mountain area is controlled by water regime at the time of vessel expansion, and this control was strongly modulated by soil characteristics. However, the correct identification and understanding of the signal was done through a detailed optimization of the data set, because not all vessels were recording the same environmental factor, nor with the same intensity. Only a careful analysis of vessels subsets by progressive filtering (Campelo et al. 2010; García-González and Fonti 2006) and the analysis of vessel rows (González-González et al. 2014) did provide a

complete picture of the ecological significance of the chronologies of earlywood vessel size. Our results confirm the sensitivity of vessel chronologies as reliable proxies recording both regional and local ecological conditions, but its use for more relevant issues such as global change studies (Fonti et al. 2010) requires a detailed optimization of vessel data.

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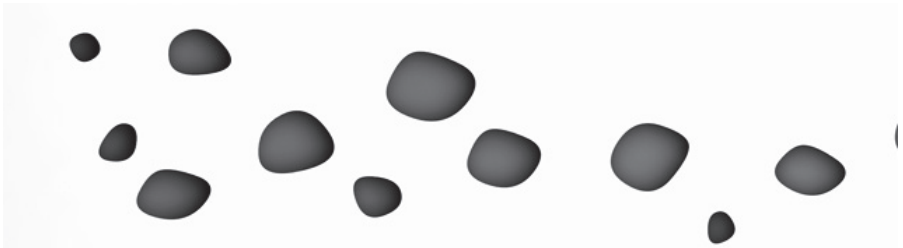
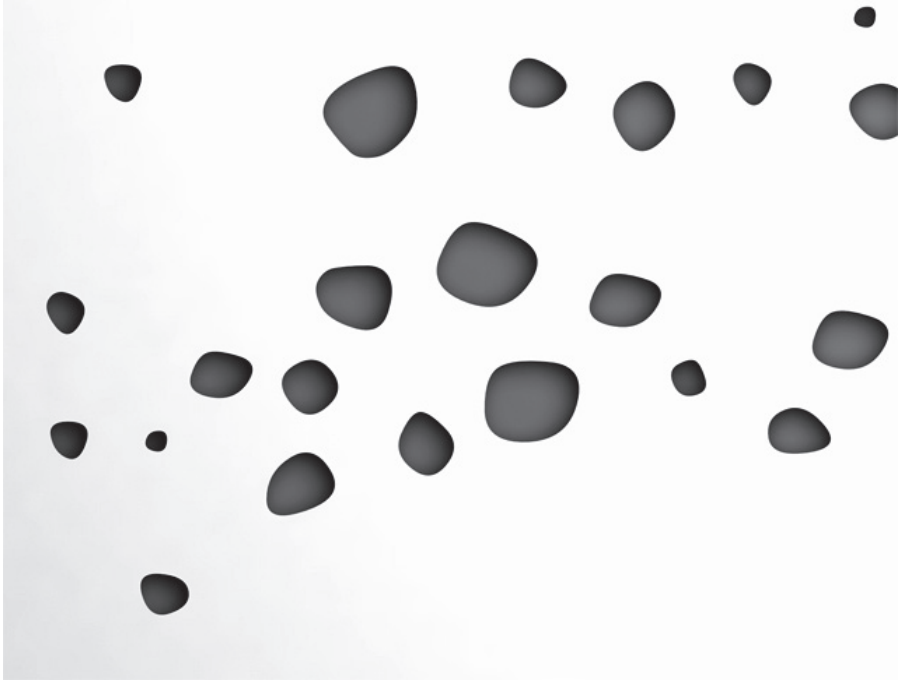


ARTICLE III. DENDROCHRONOLOGICAL ANALYSIS OF THE ADAPTATIONS OF EARLYWOOD VESSELS OF* QUERCUS ROBUR *L. ALONG AN ELEVATIONAL GRADIENT IN THE NORTHWESTERN IBERIAN PENINSULA



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Abstract

We obtained four tree-ring chronologies for pedunculate oak (*Quercus robur* L.) in the northwestern Iberian Peninsula along an altitudinal gradient covering most of the species range within the region. Climate of the study area is Atlantic, with a rainfall maximum during autumn-winter, and a minimum during summer, but without the existence of a remarkable summer drought. We measured the earlywood vessels and the width for each ring, and obtained chronologies of several growth variables, which were compared to each other. Then, we selected a subset of six earlywood variables and latewood width for further analysis. The period considered was 1954-2003.

There was a clear discrimination between the lowest site and the others, due to milder climate conditions, and probably also because of soil characteristics, whereas the other sites tended to behave within a gradient. Earlywood and latewood were unrelated, and controlled by different factors, except at the lowest site, where the response was stronger. A considerable number of variables was necessary to study earlywood, and showed that it was usually related to conditions during its formation, whereas a certain effect of the previous or dormant season was only observed at the lowest sites; Water availability during late spring and summer affected earlywood width, but only at low elevation.

Despite obtaining reliable results, the present paper illustrates the complexity of analyzing climate-growth relationships in oceanic areas under the absence of a prevailing limiting factor.

Keywords: Pedunculate oak, tree ring, quantitative wood anatomy, Atlantic climate**,** climate-growth relationships

Introduction

Quercus robur, together with *Q. petraea*, is one of the most widespread species in European forests. It dominates forests from sub-boreal to sub-Mediterranean environments, forming monospecific forests, or mixed with other species (Ellenberg 2009). It is therefore a key species, responsible for a high biodiversity, and also of important economic value. It reaches southwestern distribution boundary in contact to the Mediterranean region in the northern Iberian Peninsula (Sánchez-de-Dios et al. 2009), with *Q. robur* covering most western areas, whereas *Q. petraea* occurs in the northeast of the Cantabrian Range (Amaral Franco 1990). But species distribution is dynamic, and is modified along with climate changes (Walther et al. 2002). Therefore, the more limiting conditions predicted for southern Europe (IPCC 2013) could affect the performance of these temperate oak species, becoming relegated to some enclaves in the northern mountains of the Iberian Peninsula (Benito Garzón et al. 2008).

Galicia, in northwestern Iberia, is characterized by a climatic regime dominated by Atlantic conditions, but with an increasing effect of summer drought towards the south and inland in transition to the Mediterranean region (Martínez Cortizas et al. 1999a). Although temperate oak forests dominate the natural vegetation in this area, they are soon replaced by the sub-Mediterranean *Q. pyrenaica* (Díaz-Maroto and Vila-Lameiro 2007). But due to the complex variation of temperature and precipitation regimes (Martínez Cortizas et al. 1999b), different types of oak forests are present in the region (Rivas-Martínez 1987; Rivas-Martínez et al. 2001). Whereas summer conditions are dry towards the inland and the Atlantic, northern Galician oak forests are influenced by milder conditions, with abundant moisture during summer time, and warm temperature for most of the year; as a result, numerous ferns and other moisture-tolerant species are present, accompanied by thermophile plants at low elevation, including relict Macaronesian elements (Izco et al. 1990).

The response of forest ecosystems to drought, as is the case in the limit of the Mediterranean region, has been the object of many investigations (Benito Garzón et al. 2008; Sánchez-de-Dios et al. 2009; Shestakova et al. 2014; Vicente-Serrano et al. 2014). One of the

most employed methods is the analysis of tree-ring chronologies (de Luis et al. 2013; Granda et al. 2013; Rozas et al. 2015), because they allow studying the whole life history of the trees. Total ring width is the parameter mostly used to establish climate-growth relationships in dendrochronology, but recently wood anatomical analysis proved to be a valuable tool to analyze the response of trees under changing environmental conditions (Fonti et al. 2010), because it allow gaining additional information. Particularly, the large earlywood vessels of ring-porous trees like oak resulted to be a very powerful proxy, which were used not only to understand the responses of trees in the Atlantic-Mediterranean transition (González-González et al. 2014), but also in areas lacking a prevailing climatic limitation (Fonti and García-González 2008). One of the main advantages of using anatomical features is that they are a link to tree physiology, as they are closely related to hydraulic properties (Fonti et al. 2010; Gea-Izquierdo et al. 2012). Therefore, their analysis appears to be suitable to understand the climatic behavior of moist oak forests in the northwestern Iberian Peninsula.

Ring-porous trees, as is the case for oak, form large earlywood vessels for an efficient water transport at the beginning of season, whereas summer growth mainly consists in structural tissues and much smaller vessels, when water conditions can be more limiting (Aloni 2015; Carlquist 1988). As a result, discrimination between earlywood and latewood is straightforward, and there are many evidences that both tree-ring compartments account for a different environmental control (Fonti et al. 2010; García-González and Eckstein 2003; González-González et al. 2014). While earlywood is rather different, ring width and latewood width are very close to each other (González-González et al. 2014; Rozas et al. 2009), but in some cases the single analysis of latewood provided more information on tree responses (Rozas and García-González 2012). As ring-porous trees need to form their large earlywood vessels every year because they are functional only during one season (Barbaroux and Bréda 2002; Tyree and Cochard 1996), latewood width appears to be a better descriptor of radial growth that latewood width itself, so that earlywood and latewood should be studied separately (Kern et al. 2013). On the other hand, earlywood characteristics, mainy vessels size, proved to be a source of ecologically relevant information. Many works dealing with this anatomical trait were limited to the influence of climate one or a few nearby sites (Fonti and García-González 2004; García-González and Eckstein 2003), or the response of sympatric species (González-González et al. 2014; González-González et al. 2015). However, the amount of investigations along gradients and/or over wide geographical areas are more scarce (Fonti and García-González 2008; Gea-Izquierdo et al. 2012; Matisons et al. 2012).

In this paper, we aim at understanding the climatic control of radial growth and wood formation in oaks (*Q. robur*) growing in the northwestern Iberian Peninsula outside the transition to the Mediterranean region, by using earlywood anatomical traits and latewood increment. To our knowledge, no research of this kind involving dendrochronology of earlywood vessels has been carried out along a temperature gradient, in an area where precipitation is apparently not limiting. Our main objectives are i) to determine the potential of the different growth variables to analyze climate-growth relationships, and ii) to identify how climate is affecting the performance of species along the gradient.

Materials and Methods

Study area and sites

We studied four pedunculate oak (*Quercus robur*) stands along an increasing elevation gradient towards inland in the northwestern Iberian Peninsula (42.80-43.64 ºN, 6.87-8.04 ºW), from the northern coastline to the Cantabrian Range, which constitutes the whole altitudinal range of the species in the region (Fig. 1). The area is dominated by an Atlantic climatic regime, with a high rainfall (1,000-2,000 mm) mainly concentrated autumn and winter due to the low-pressure system over the ocean, and clearly increasing along with elevation. There is in general no remarkable summer drought, as

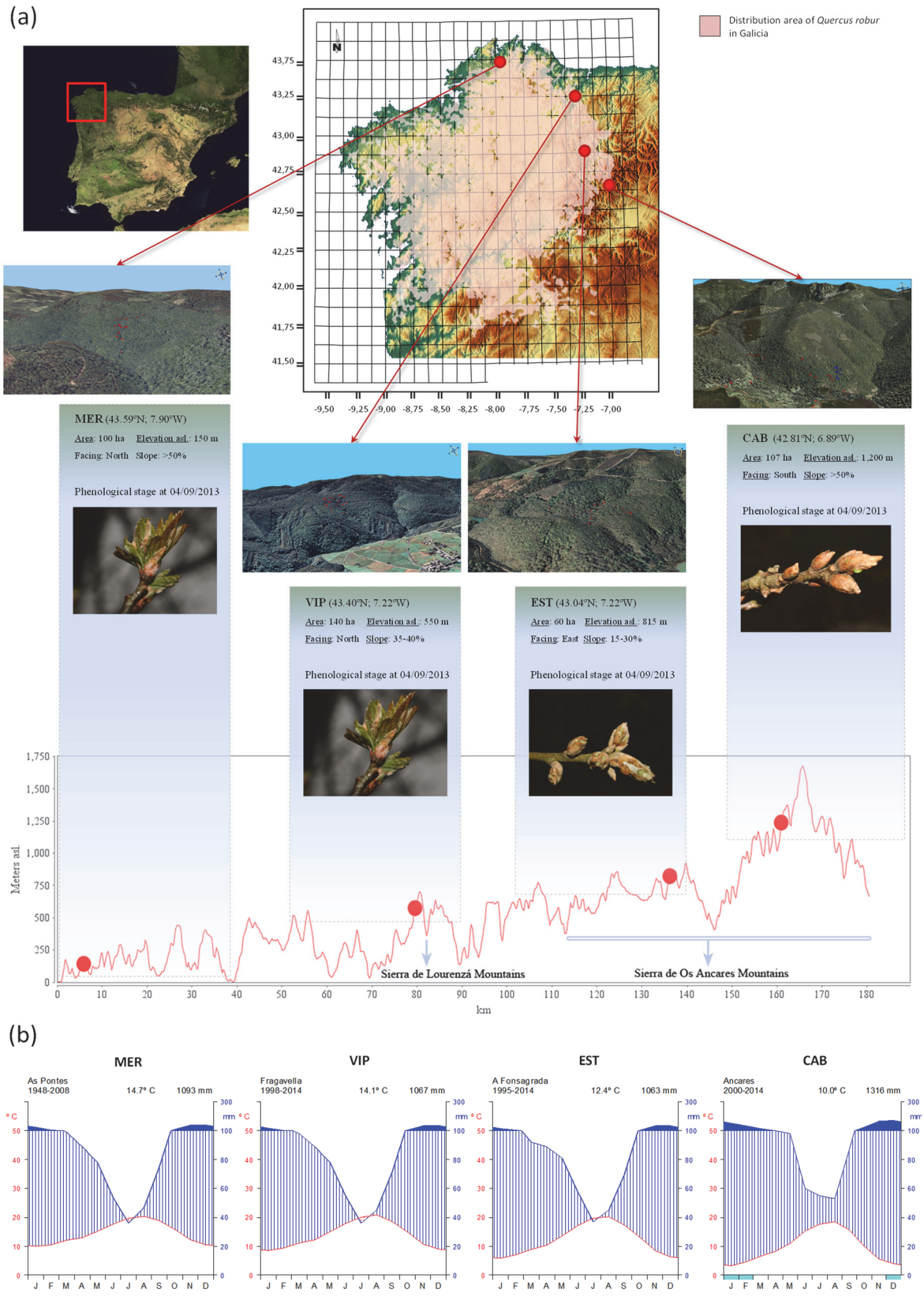

Fig. 1. Location of the four chronology sites along the altitudinal gradient (a), showing a topographic profile, and the physiographic characterization of each one. Digital Terrain Model (DTM), with sampled trees as red points, and relevant phenological information for 2013 are shown, as well as corresponding climate diagram from nearby weather stations (b).

northern winds carry high humidity to low-elevation coastal areas, and frequent summer storms palliate drought in the mountains. Annual temperature considerably varies along the gradient (13 ºC at MER, down to 8 ºC at CAB), with very mild conditions and nearly no frost risk near the coastline, whereas temperature decreases, and thermal oscillation and continentality increase to the inland, resulting in long frost and snow periods during winter (Table 2). Consequently, the growing season is much longer at the northernmost sites, while the period of active growth at high elevation can span for only five to six months a year.

All stands (Table 1) are clearly located within Eurosiberian region (Rodríguez Guitián and Ramil Rego 2008). Sites below 1,000 m asl. (MER, VIP, and EST) belong to the association *Blechno spicanti-Quercetum roboris* (Rivas-Martínez 1987); they are natural forests dominated by pedunculate oak together with *Castanea sativa* Miller., *Betula alba* L. and *Acer pseudoplatanus* L., having in general a mild temperature and a high moisture throughout the year, which favors the presence of characteristic understory species such as *Vaccinium myrtillus* L., and a notably abundance and diversity of ferns as *Blechnum spicant* L. On the other hand, CAB fits into the association *Linario triornithophorae-Quercetum petraeae* (Rivas-Martínez 1987), a mountain forest where oak is accompanied by several other trees species (e.g., *Betula alba*, *Acer pseudoplatanus, Sorbus aucuparia* L., *Corylus avellana* L*., and Ilex aquifolium* L.).

The lowest site MER is located on a slope facing north near the coastline, and dominated by unmanaged and young oak trees. The abundance of thermophile species indicates the mild climatic conditions of the stand, with small trees and shrubs such as *Laurus nobilis* L., *Arbutus unedo* L. or the understory *Ruscus aculeatus* L. The lack of drought facilitates the presence of ferns as *Blechnum spicant*, *Dryopteris affinis* (Lowe) Fraser-Jenk., *D. dilatata*, (Hoffm.) A. Gray or even Macaronesian elements as *Davallia canariensis* (L.) Sm., or *Woodwardia radicans* (L.) Sm. linked to watercourses. This is practically the only extent woodland that can be found in this low-elevation area, where most of the natural distribution area has been replaced by *Eucalyptus* plantations.

VIP lies at a higher elevation, and it also constitutes one of the few well-preserved and scarcely managed oak woodlands at present. Is is a young forest on deep clay soil that still maintains a remarkable coastal influence. Oak is mixed with *Castanea sativa* in the main canopy over; other companion tree species found are *Salix* sp., *Corylus avellana* and *Betula alba*, with *Alnus glutinosa* L. and *Populus nigra* L. towards the valley. Fern species (*Blechnum spicant*, *Dryopteris filix-mas* L.) are still abundant, but most thermophile elements do not occur.

EST is also located at mid-elevation, but climate is more continental, even with frequent frost and snow that occur very occasionally at VIP. It is dominated by medium-size oak trees, frequently intermingled with *Betula alba*, and with the occasional presence of other trees as *Fagus sylvativa* L., *Q. pyrenaica* or *Taxus baccata* L., *Corylus avellana*, *Prunus spinosa* L., *Sorbus aucuparia*, or *Ilex aquifolium*. *Blechnum spicant* and *Vaccinium myrtillus* are abundant in the understory, but species diversity is considerably lower than at the former sites.

Table 1. Physiographic characterization of the study sites.

Location	Code	Area (ha)	Elevation (m)	Facing	Slope (%)	Latitude (ºN)	Longitude (ºW)
Fraga dos Casás	**MER**	100	150	North	>50	43.59	7.90
Vilapena	**VIP**	140	550	North	35-40	43.40	7.22
Estornín	**EST**	65	815	East	15-30	43.04	7.22
Cabanavella	**CAB**	107	1200	Southwest	>50	42.81	6.89

CAB is close to the altitudinal limit of the species Trees grown on a steep slope within a large forest dominated by *Quercus robur*/*Q. petraea* and their potential hybrids. Ancient oaks are scattered within younger trees, and under very low human disturbance nowadays. Most sampled oaks grew on a south-southwest slope, where the tree layer was almost monospecific, except for more tree species only present in shady areas (e.g., *Fraxinus excelsior* L., *Acer pseudoplatanus*, *Betula alba* or *Ilex aquifolium*), and with a very poor understory.

Sampling, processing, and anatomical measurements

We extracted at least two 5-mm increment cores at breast height from 22 to 45 dominant trees at each site. Extracted cores were air-dried, glued onto wooden mounts for easy handling, and submitted to several processes to ensure an optimal visualization of tree rings and vessel elements. We first proceeded by cutting a regular cross-sectional surface using a WSL sliding microtome (Gärtner and Nievergelt 2010), followed by manual polishing with progressively finer sandpaper (grain sizes from P220 to P1200, FEPA (Federation of European Producers of Abrasives) Abrasives). Due to the hardness of wood, microtome cutting just ensured that the core surface was even enough for vessel observation, but it was necessary to finish its preparation by sanding. We removed tyloses and wood dust inside earlywood vessel lumina using high-pressure water blast (Fonti et al. 2002). Once dry, samples were stained with black printer ink, and earlywood vessels filled with chalk dust (González-González et al. 2014), finally achieving an optimal contrast for image analysis.

We measured earlywood (EW) and latewood (LW) widths to the nearest 0.001 mm with a tree-ring measuring device linear stage (Velmex TA UniSlide, Velmex Inc., Bloomfield NY, USA) supplemented with a binocular microscope (Olympus SZ60) at 20-40x magnification. The boundary between earlywood and latewood was determined by vessel size and wood structure (García-González and Eckstein 2003); large vessels formed a continuous band of earlywood, and flamelike groups of smaller conducting elements and parenchyma were scattered within a fiber matrix in the latewood. We checked crossdating on the series of total ring width (RW), calculated as the sum of EW and LW, by first comparing individual curves, and then using COFECHA (Grissino-Mayer 2001) for statistical verification.

Table 2. Climatic characterization of the study sites.

Sites	MER	VIP	EST	CAB
Annual Mean Temperature (ºC)	13.2	11.7	10.1	8.0
Summer Mean Temperature (ºC)	17.6	17.1	16.3	14.0
Winter Mean Temperature (ºC)	8.6	7.9	5.8	1.6
Annual Mean precipitation (mm)	1365.0	964.2	1312.6	1912.8
Summer Mean precipitation (mm)	43.7	31.9	44.8	59.6
Annual Water Balance (mm)	602.9	283.1	664.6	1390.5
Summer Water Balance (mm)	-73.2	-74.8	-45.7	-20.6
Climate description	Winters mild, short, no frost period. Summers cold. Fogs with high moisture, weak temperature range	Winters mild without frost period. Summers cold. Low temperature range	Winters cold, with dale mists, thermal inversion and occasional snowfalls. Mild summers	Winters very cold, with frosts, snowfalls and dale mists. Mild summers with frequent storm rainfall

Vessel measurements were taken from a subset of 12 to17 trees per site, selected basing on characteristics as high correlation with the local chronology, low incidence of growth constraints or anomalies, or safe dating. These cores were digitized using a device integrated by a digital photo camera Canon EOS 600D, coupled to a binocular microscope Olympus SZ60, and a mechanically driven platform. We obtained sequences of high resolution images (5184 x 3456, 17.9 Mpx) across the whole core, and partially overlapped. We stitched consecutive images using the program PTGui ver. 9.1.8 Pro (New House Internet Services B.V., Rotterdam, The Netherlands), and saved the resulting images into Tagged Image File Format (TIFF) files to prevent loss of quality. In order to avoid potential distortions by this procedure, images were overlapped over a high distance (at least 30%), and areas with apparent distortion in the images were discarded.

Earlywood vessels were automatically recognized and measured in ImageJ (Abramoff et al. 2004; Schneider et al. 2012) using the VesselJ plugin (García-González, not published). Using an 8-bit (gray) threshold level, bright vessel lumina were detected due to the contrast with the dark background tissue. However, a correct recognition of all vessels was hindered by the occasional presence of dust spots or other defects, and we optimized this process by filtering out the objects detected by the image analysis system that did not appear to be vessels. For this, we fixed minimum vessel area (size filter) of 10,000 μm^2 (Eilmann et al. 2006; Fonti and García-González 2004), and used circularity (shape filter) to remove other objects more than twice larger than wide. But we still needed some manual corrections on the image to properly identify all conductive elements in many cases, as not all objects other than vessels could be excluded by the filters. Finally, once all vessels had been correctly detected, we applied morphological operations (erode-dilate 2x2 one pass, and calculation of the convex hull) to improve vessel outlines before saving area values, outlines, and object coordinates.

Measured vessels were allocated to the corresponding tree rings by Autovasos (García González, not published), a software specifically designed for ring-porous species. This software also allowed us to check if vessel dating was correct, by comparing the measured and previously crossdated curves to the estimates of tree-ring width provided by Autovasos. In a last step, earlywood vessels within each tree ring were separated into two groups, distinguishing between i) those formed at the beginning of growing season, located on first row which usually offer a higher sensitivity to climate (García-González and Fonti 2008), and ii) the rest of vessels formed later in the season. We considered that the first row should include all vessels located immediately after the ring boundary, and also those whose beginning did not exceed the imaginary line connecting the centers of the vessels on the ring boundary.

Chronologies of wood-anatomical and growth variables

We pooled all measurements from increment cores of the same tree into a single data set for each ring. Consequently, we initially calculated several earlywood variables from the vessel data, and built annual time series for each one. These variables were: total number of vessels (NV), mean vessel area (MVA), mean vessel area of vessels larger than 90th percentile of each ring (M90), total vessel area (TVA), hydraulically-weighted diameter for all measured vessels (HYD = $\sum_1^n d_t^5 \,/\, \sum_1^n d_t^4$, for n vessels of diameter d in year t), which is proportional to xylem hydraulic conductivity (Fichot et al. 2009; Sperry et al. 1994), and the specific conductivity (KS) of the earlywood (kg m^{-1} s^{-1} MPa^{-1}) in year t according to the Hagen-Poiseuille law (Tyree and Zimmermann 2002), so that KS = $(\pi\varrho/128\eta A)\sum_1^n d_t^4$, where ϱ is the density of water at 20 ºC (998.2 kg m^{-3}), η is the viscosity of water at 20 ºC (1.002 x 10 $^{-9}$ MPa), A is the area of the earlywood analyzed in yeat t, and d_t is the diameter of each of the n vessels measured in year t. NV and TVA were standardized to a frame of 10 mm of tangential width, whereas conductive to obtain KS was automatically calculated from the convex polygon including all vessels. We also calculated size-related variables (MVA, M90, and HYD) for vessels only in first row (*r1*), as well as for vessels not

located on that row (*nr1*). This separation was necessary, because the largest vessels on the ring boundary, formed at the beginning of the growing season, usually optimize the climatic signal (García-González and Fonti 2006). Besides the anatomical variables, RW, EW, and LW were also included in the analyses, yielding a total set of 15 variables.

Growth-related trends were removed from the series by fitting a cubic smoothing spline function (Cook 1990) with 32-years stiffness and 50% cutoff, and dividing by the value estimated by the function. This spline should mainly retain interannual variability, minimizing ontogenetic and disturbance signals in tree-ring growth. We applied the same detrending method to all variables to ensure that differences in statistical procedures did not influence the final result. After detrending, the obtained time series of growth indices was averaged into a chronology using a biweight robust mean (Mosteller and Tukey 1977) to minimize the effects of outliers. These procedures were applied to each variable, and site, resulting in 60 different mean chronologies.

As this amount of variables was excessive to carry out and display all results, and many of them should bear a high collinearity, we optimized the number of study variables by principal component analysis (PCA), a method successfully applied by other authors to identify groups of variables with similar information (Bryukhanova and Fonti 2013; González-González et al. 2014). For each site, PCA was performed on the correlation matrix of all chronologies, and we used the ordination on the space defined by the two first eigenvectors (following varimax rotation) to identify groups of related variables. As correlation within each identified group was very high, we based the analysis of earlywood on a set of six variables, grouped in three subsets, and we checked their behavior within each plot and throughout the gradient. Thus, were studied TVA and NV, MVA and KS, and hydraulic diameter separated by rows (HYD-r1, HYD-nr1). The characterization of the radial growth was performed by means of LW. PCA was also used to analyze the relationships among sites across the gradient for each study variable.

Individual series and chronologies were characterized by standard dendrochronological statistics, including the mean sensitivity (*MS*), which measures year-to-year variability; and the first order autocorrelation coefficient (*AR1*), a measure of the influence of the previous year. We also assessed the statistical quality of each single chronology, i.e., its 'common signal' following Cook et al. (1992). We computed the mean value of all cross-correlations between trees (*Rbt*), and also estimated how representative the sample was as compared to an infinite population, a statistic known as expressed population signal (*EPS*). The period for all analyses was 1954-2003 (50 years), because it was covered by at least eight trees at each site.

Climate-growth relationships

We obtained climate-growth relationships by calculating Pearson's correlations between the chronologies and climatic data along the growing season. Confidence intervals for the correlation coefficients were assessed by 10,000 bootstrap iterations, applying the correction proposed by Mason and Mimmack (1992) for the percentile confidence intervals. The period for the analysis was the same as for chronology comparison and assessment (1954-2003).

The analyzed growth variables were LW for latewood, and NV, TVA, MVA, KS, HYD-r1, and HYD-nr1 for earlywood. The time window for the growing season was from previous May to current May for earlywood variables, and extended to current September for latewood. Apart from analyzing the responses for single months, climate records were seasonalized into critical periods for tree growth, namely dormancy (December-February), and quiescence-reactivation (February-April) for earlywood variables; and early summer (June-July) for latewood.

Due to the lack of reliable local records, climatic data consisted of gridded CRU TS 3.22 data (University of East Anglia Climatic Research Unit et al. 2014) of monthly precipitation and temperature (mean, minimum, and maximum), which were taken from the KNMI Climate Explorer (http://climexp.knmi.nl/) for the

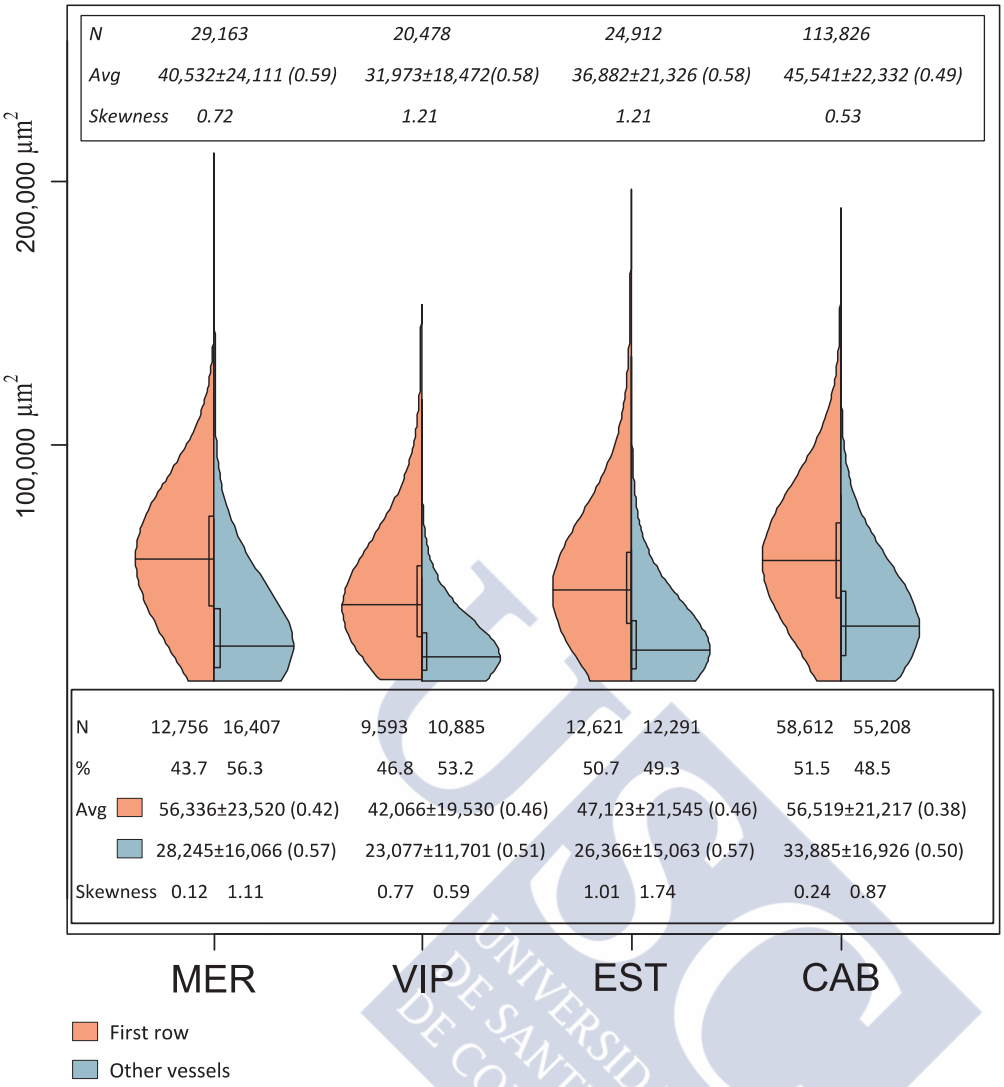

Fig. 2. Violin plots showing vessel distribution at each site, with their corresponding descriptive statistics, for all measured vessels (italics, upper), and for the vessels separated by rows (lower). *N*: number of vessels; *Avg*: mean vessel area (µm^2) ± standard deviation (coefficient of variation).

period 1901-2013, with 0.5º of spatial resolution.

Results

Vessel-size distribution

We measured 188,379 earlywood vessels. More than the half belonged to CAB (Fig. 2), due to the greater number of selected trees (17), and the much longer chronology (1523-2003). Sites located at the limits of the altitudinal gradient (MER and CAB) showed the highest mean values of vessel size, considering either all measured vessels or separated rows; they were similar for the first row (56,519 for CAB, and 56,336 µm^2 for MER), but notably larger at CAB for those not located in the first row (33,885 for CAB, and28,245 µm^2 for MER). The intermediate elevation stands (VIP and EST) registered lower values for all groups of vessels. There was also an increasing trend along with elevation except for MER.

The predominance of small vessels within the tree ring was evident for all sites, but especially for the mid-elevation VIP and EST (skewness coefficient was 1.21 for both, and below 1.0 for the other sites). In fact, the asymmetrical distribution found when separating vessels

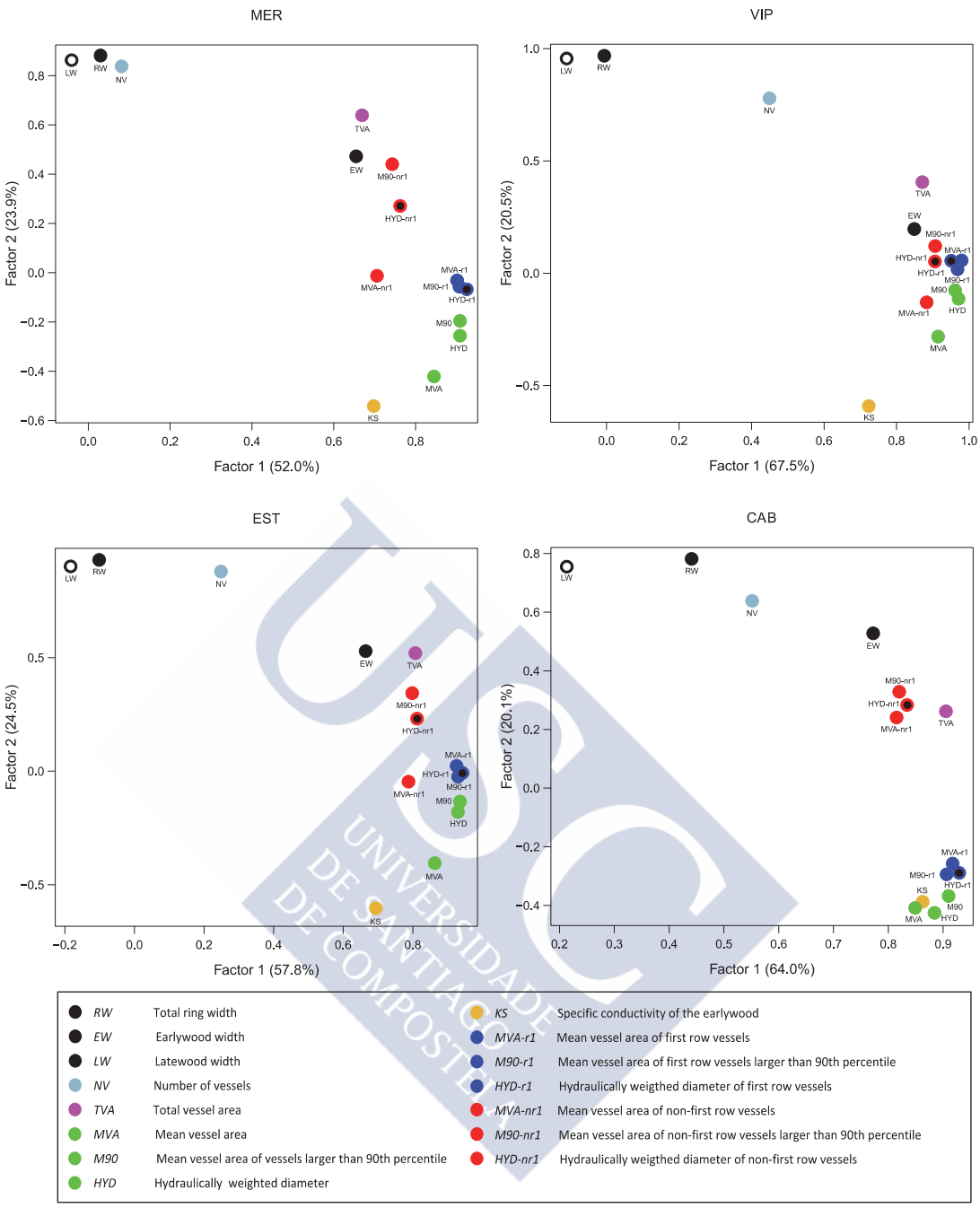

Fig. 3. Principal component analysis (PCA) of the preliminary study variables along the period 1954-2003 for all chronologies, following varimax rotation. Double dots correspond to the selected variables.

suggested a considerable presence of small ones not only for the latest rows (skewness 1.59 and 1.74, respectively), but also for first row (0.77 and 1.01). This was not the situation for MER and CAB, because although the distribution of vessels formed later in the season was also skewed towards the smallest sizes, but less than for mid-elevation, the distribution of the first row tended to be normal, especially at MER. The ratio of CAB, because although the distribution of vessels formed later in the season was also skewed towards the smallest sizes, but less than for mid-elevation, the distribution of the first row tended to be normal, especially at MER. The ratio of vessels belonging to the first row was in general slightly less than the half (43.7% to 51.5%), and increased with elevation, but age structure has not been taken into account.

Selection of variables

Principal component analysis performed on the initial set of 15 variables tended to identify three different groups, which should correspond to different kinds of ecological information (Fig. 3). The general pattern was very similar, but a few variables slightly varied in their grouping depending on the site.

A first subset comprised RW and LW, together with NV, i.e, the variables most related to the number of cells produced; NV was clearly very close to the width variables at MER, but more intermediate at VIP and EST. This first group was clearly separated from all other anatomical variables (which were to some extent dependent on vessel size) along the first eigenvector. However, these other variables were clearly ordinated along the second eigenvector, and were even prone to split into two subgroups. One of them, in the upper part of the axis, included EW, TVA, and size variables of the vessels not located in the first row (MVA-nr1, HYD-nr1, M90-nr1), whereas the second subgroup included the size variables for all vessels (MVA, HYD, M90), and for the first row (MVA-r1, HYD-r1, M90-r1); KS was also ordinated with these variables, but less related to them except for CAB.

The ordination of the vessel variables presented well-defined groups for CAB, whereas this behavior along the second eigenvector was very weak at VIP, indicating that most variables shared a great amount of common information. MER at EST showed a clearer ordination, but not a remarkable splitting into groups as found for CAB.

In view of these results, the selection of variables by PCA analysis was not straightforward, and therefore we decided to still keep a considerable number of them for the analysis of chronology assessment and climate-growth relationships. Thus, we selected six different variables for earlywood. First, we included those separated at both sides of the second eigenvector (NV and TVA vs. KS and MVA). NV and KS were the variables apparently showing less similarities, whereas TVA was close to NV, though more similar to the rest of the earlywood than NV itself, and so did MVA in comparison to KS. In addition, we considered two variables (one for each vessel row) that seemed to be somehow different, namely HYD-r1 and HYD-nr1; this selection was especially remarkable for the case of CAB. LW was also added to the final set of variables, characterizing thereby the increment of the ring.

Analysis of chronologies

Earlywood variables presented in general a low common signal (Table 3), and no remarkable differences among variables, with the exception of NV (*Rbt*=0.12-0.16) and HYD-nr1, which had a very low value at MER (*Rbt*=0.08). The other earlywood variables showed similar statistics (*Rbt*=0.12-0.34, *EPS*=0.51-0.84). Differences among sites were remarkable, with the lowest common signal at MER for all variables, whereas the three other sites were not very different from each other; however, the highest common signal was recorded at VIP. Year-to-year variability, as expressed by MS, was also low for all earlywood parameters (0.06-0.22), especially for those variables only derived of vessel size (MVA, HYD-r1, HYD-nr1), which did not reach 0.10 at any site. As regards autocorrelation, there was only a weak influence of previous year growth at MER (*AR1*=0.10-0.35). The best statistical quality was observed for the LW chronologies, with a high common signal (*Rbt*>0.35 and *EPS*>0.85, values recomended by Wigley et al. (1984), at sites located in the eastern inland mountains, but MER exhibited a much lower signal, though close to these thresholds. Year-to-year variability increased gradually along with elevation (from 0.22 at MER up to 0.40 at CAB). Detrended LW series were not autocorrelated.

Ordination by PCA for all study variables displayed in general a clear separation of MER (Fig. 4). Whereas loadings on the first eigenvector ranged 0.6-0.9 for the other three sites (EST, VIP, and CAB), the value was close to 0 for MER, regardless of the variable considered,except for HYD-nr1. In respect of the other sites, there was in general a trend to ordinate following the altitudinal gradient, but it was not the case for all variables. This pattern

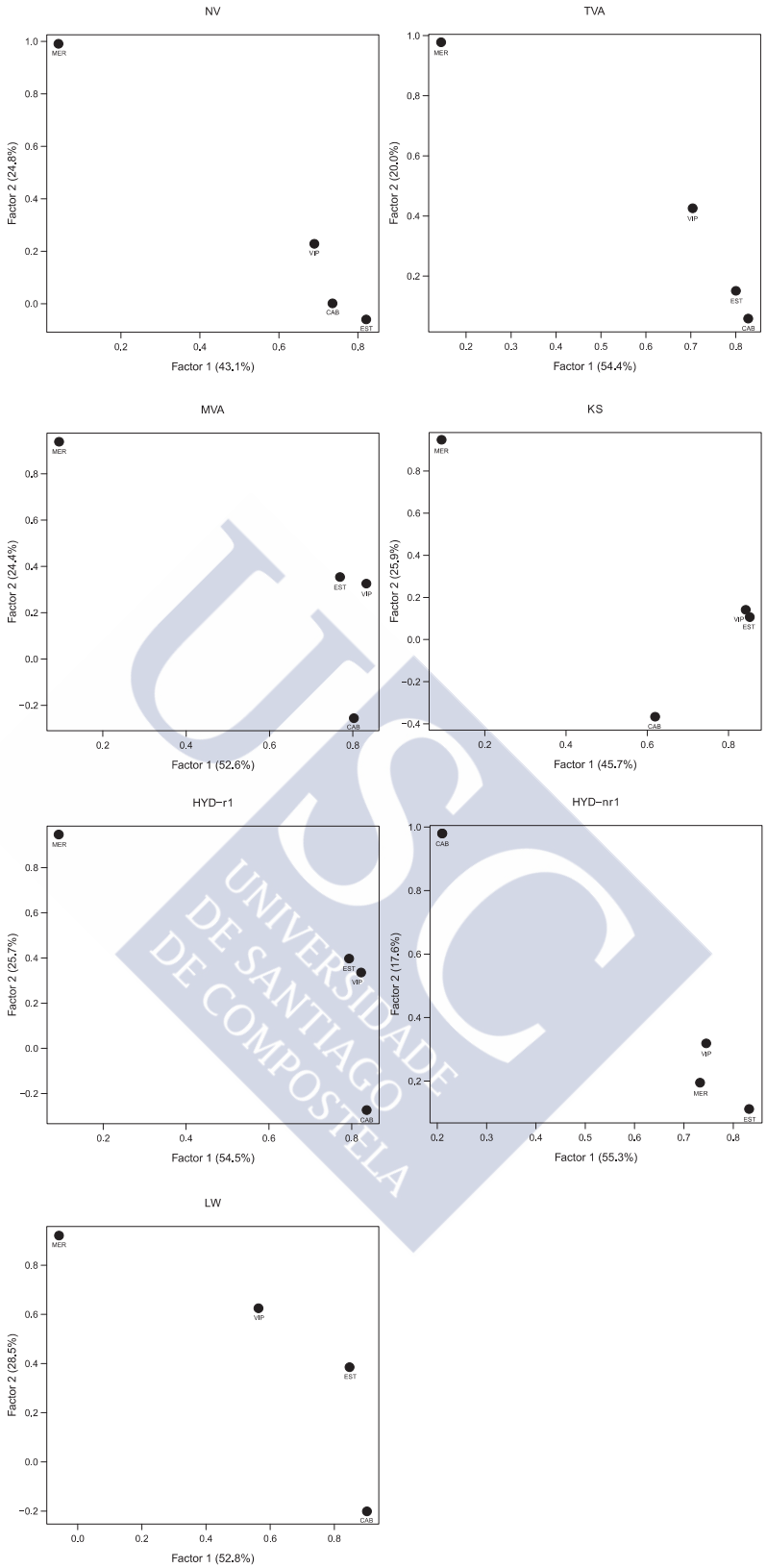

Fig. 4. Principal component analysis showing the ordination of sites on the space defined by the two first eigenvectors, following varimax rotation along the period 1954-2003. LW: latewood width; NV: number of vessels; TVA: total vessel area; KS: specific conductivity; HYD-r1: hydraulic diameter for the first row; HYD-nr1: hydraulic diameter for vessels not in the first row.

Table 3. Assessment of chronology quality for the common interval 1954-2003. Total covered period was 1936-2012 (77 years) at MER, 1938-2007 (70 years) at VIP, 1908-2007 (100 years) at EST, and 1523-2003 (481 years) at CAB. Variables considered were NV (number of vessels), TVA (total vessel area), MVA (mean vessel area), KS (specific conductivity), HYD-r1 (hydraulically weighted diameter of first row vessels), HYD-nr1 (hydraulically weighted diameter of vessels not in the first row), and LW (latewood width). *Rbt*: mean correlation between trees; *EPS*: expressed population signal; *MS*: mean sensitivity; *AR1*: first order autocorrelation coefficient.

Chrono	NV				TVA			
Plot	MER	VIP	EST	CAB	MER	VIP	EST	CAB
Rbt	0.12	0.16	0.15	0.16	0.12	0.26	0.21	0.24
EPS	0.52	0.65	0.68	0.76	0.51	0.78	0.76	0.84
MS	0.09	0.12	0.11	0.09	0.09	0.15	0.11	0.12
AR1	0.30	-0.02	-0.06	-0.18	0.35	-0.07	0.04	-0.15

Chrono	MVA				KS			
Plot	MER	VIP	EST	CAB	MER	VIP	EST	CAB
Rbt	0.20	0.33	0.23	0.22	0.16	0.29	0.25	0.19
EPS	0.67	0.83	0.78	0.82	0.60	0.81	0.80	0.80
MS	0.06	0.09	0.08	0.07	0.13	0.22	0.17	0.11
AR1	0.29	0.03	0.00	-0.04	0.26	0.07	0.05	-0.07

Chrono	HYD-r1				HYD-nr1			
Plot	MER	VIP	EST	CAB	MER	VIP	EST	CAB
Rbt	0.18	0.34	0.26	0.26	0.08	0.29	0.13	0.19
EPS	0.63	0.84	0.81	0.85	0.42	0.80	0.63	0.79
MS	0.03	0.06	0.04	0.04	0.03	0.08	0.05	0.05
AR1	0.10	-0.02	-0.07	-0.16	0.31	-0.18	-0.14	-0.07

Chrono	LW			
Plot	MER	VIP	EST	CAB
Rbt	0.34	0.45	0.57	0.46
EPS	0.81	0.89	0.94	0.93
MS	0.22	0.27	0.37	0.40
AR1	0.06	0.08	-0.10	0.03

was clear for LW and TVA, but most earlywood variables (MVA, KS, and HYD-r1) tended to separate CAB from the two mid-elevation sites (VIP and EST), nearly showing no differences between the two latter. NV clearly splitted MER, but had with no clear pattern for the other sites. As regards HYD-nr1, it was CAB that was discriminated, probably due to the great abundance of rings formed by only one row of earlywood vessels, not observed at the rest of stands; in any case, the pattern among sites for HYD-nr1 did not resemble any of the other variables.

Climate growth-relationships

Despite the poor quality of earlywood chronologies as compared to LW, and the complexity of analyzing six different variables, their relationships to climate provided some information on the behavior of earlywood formation in most cases, as well as on differences among the study sites.

NV and TVA (Fig. 5) were considerably correlated to each other (r=0.71-0.80) and therefore close in the PCA analysis, but they had a rather different response pattern. In fact, the only common relationships were the positive role of previous summer precipitation (August) at MER, and most results at VIP. NV and TVA appeared to be related to conditions during late spring-early summer in the previous year at MER and VIP, probably due to water balance, as both precipitation (r=0.33, P<0.05 at MER; r=–0.32, P<0.01 at VIP, to last May), and temperature (mean and maximum) (r=0.27, P<0.05 with last June at MER; r=0.40, P<0.01

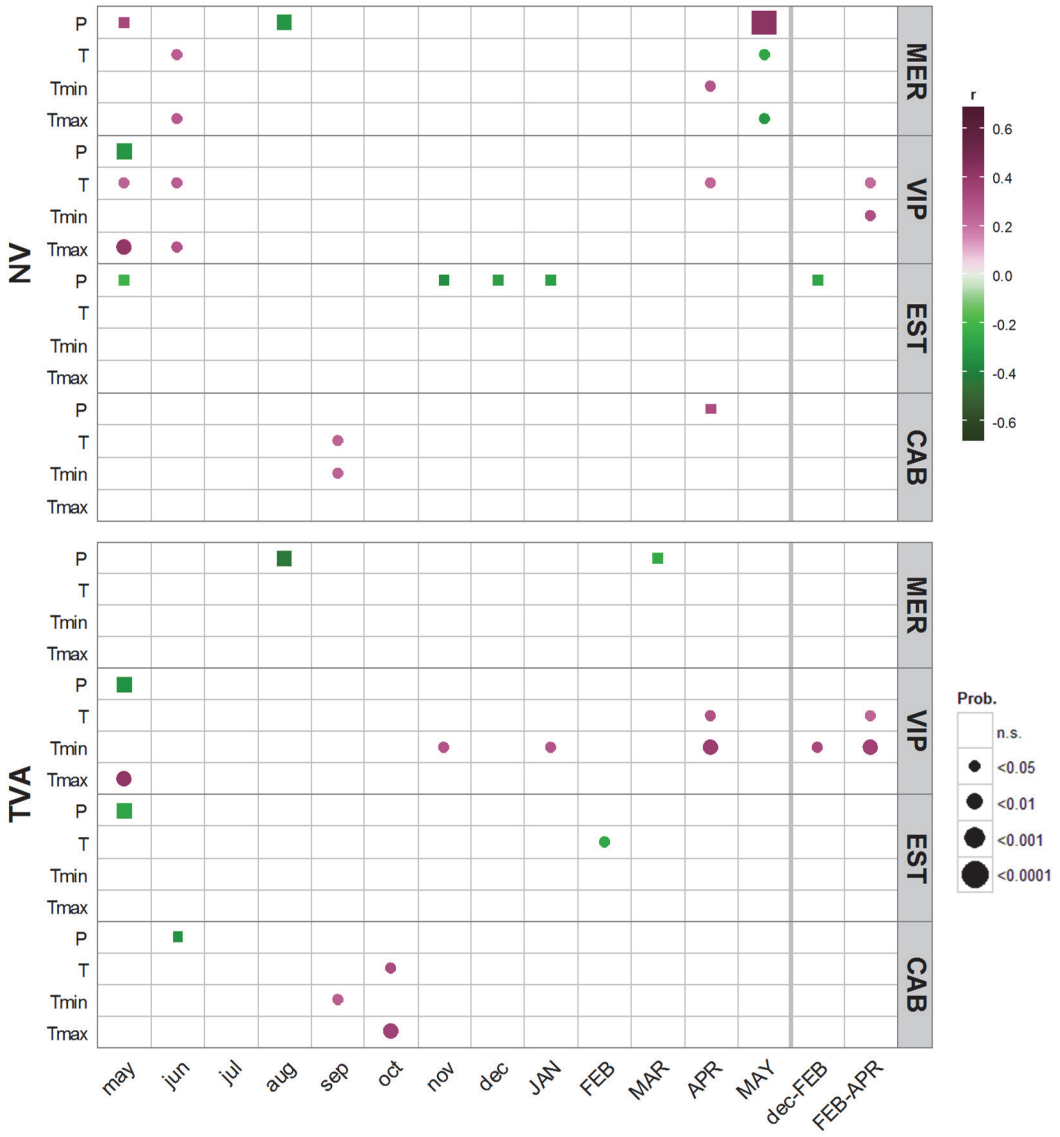

Fig. 5. Relationships to climate for the number of earlywood vessels (NV), and total vessel area (TVA), expressed as bootstrapped correlation coefficient, along the study period 1954-2003. Lowercase and uppercase letters correspond to the months of the previous and current growth year, respectively.

with last May at VIP) were correlated to NV, and with a different sign. EST also recorded the negative effect of previous summer precipitation, but the relationship was weaker. The strong positive effect of May precipitation (r=0.42, P<0.0001) at MER should also be highlighted, and this is probably due to the transition to latewood, as warmer climatic conditions at this place caused wider rings and an early differentiation of latewood.

As happened with the previous variables, the great similarity between MVA and KS (r=0.86-0.93) did not correspond to analogue climate-growth relationships in most cases (Fig. 6). They were more complex and weaker for MVA; in fact, the only remarkable relationships were spring conditions at CAB (positive response to temperature, negative to precipitation), and to a lesser extent, at MER; but these relationships were also recorded by KS, and even stronger. As regards KS, we observed a clear pattern between the lowest elevation (MER) and the highest elevation (CAB), as both sites were correlated to temperature in April, but the relationship was negative for MER (r=–0.39, P<0.01), and positive for CAB (r=0.44, P<0.01); and reversed with precipitation

(r=0.21, P<0.05; r=–0.40, P<0.01, respectively). This relation, however, was not found at any of the mid-elevation sites. The high negative correlation of May precipitation (r=–0.49, P<0.001), which contrasted with the result found for NV, was probably due to the formation of wood at the transition to the latewood, i.e., with a high number of small vessels on a large area, which implies less specific conductivity.

The analysis of HYD in the two earlywood compartments (Fig. 7) showed more remarkable results, especially as regards the first row (HYD-r1). Once again, MER was very different, and vessel size was inversely correlated to temperature during dormancy (from previous December to current February, maximized by minimum temperature, r=–0.33, P<0.01). On the contrary, it was temperature at the beginning of the growing period (April) that influenced the development of the first vessels at the other sites. We found the strongest correlation at CAB (r=0.47, P<0.01 to maximum temperature), and the signal was recorded by both maximum and minimum temperature, whereas maximized by minimum temperature at a lower elevation (VIP). The relationship at EST was less intense (r=0.35, P<0.05), but there was also a positive influence of previous fall (r=0.36, P<0.01), and a negative relation to temperature in February; no correlations to precipitation were observed.

HYD-nr1 had a weaker pattern, notably differing from that found with HYD-nr1; only VIP appeared to have the same influence of climate, which was also evidenced by the remarkably higher relationship between both vessel rows (r=0.83 for VIP, r=0.50-0.62 for the other sites, Table 4). To a lesser extend, CAB was still correlated to temperature at the onset of cambial activity (r=0.29, P<0.01). The mid-elevation sites VIP and EST showed the influence of previous summer, either to rainfall (r=0.26, P<0.05; r=–0.33, P<0.01) or to temperature (r=0.27, P<0.05; r=0.30, P<0.01), also registered by NV and TVA.

Climate-growth relationship of LW were restricted to the current growing season, and mainly from late spring to early summer (Fig. 8). But there was no spatial pattern, as they considerably differed among sites. CAB and VIP had very few and weak significant coefficients. In general, latewood formation was linked to temperature, except for MER, where the response to early summer water availability was strong, with a high positive correlation to rainfall (r=0.53, P<0.0001), and sligthly weaker to temperature (r=–0.38, P<0.01). In addition, MER had also a close relationship to spring temperature (r=0.41, P<0.001 for April), whereas this positive effect of spring temperature was weaker and delayed (May) for the mid-elevation sites VIP and EST. Finally, we also a positive relation between temperature at the end of the season (September) and LW, especially for EST (r=0.39, P<0.01), but also for CAB.

Table 4. Correlations between rows of mean vessel area (MVA) and hydraulic diameter (HYD). r1: vessels in the first row; *nr1*: vessels not belonging to the first row.

Chronos	MER	VIP	EST	CAB
MVA r1 / nr1	0.41	0.75	0.58	0.50
HYD r1 / nr1	0.50	0.83	0.62	0.61

Discussion

Comparison among site chronologies

In view of our results, the four sites selected did not result to be a proper gradient. Most variables pointed out that the three eastern sites tended to be a gradient, and both the ordination of chronologies and some relevant results of climate-growth relationships varied with elevation. But the low-elevation, western site MER completely differed from the others, even from VIP, which is also close to the coastline. Possible explanations involve differences in the climatic conditions, but also other non-climatic factors specifically linked to the site.

Due to the lower elevation and proximity to the coastline, MER is the warmest site, but temperature has a rather regular variation with altitude in the region (Martínez Cortizas et al. 1999c), and clearly follows a gradient. In

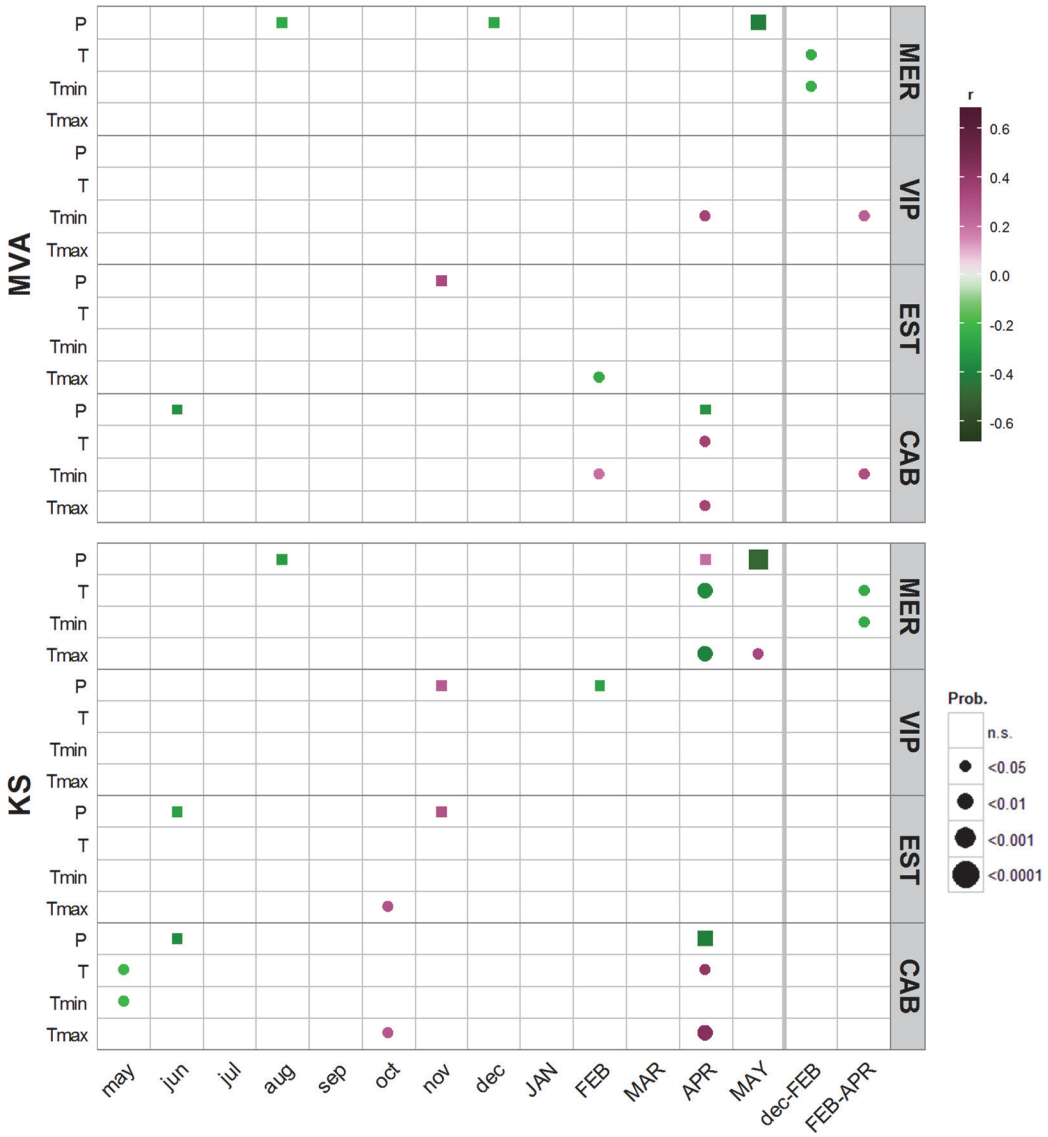

Fig. 6. Relationships to climate for mean vessel area (MVA), and specific conductivity (KS), expressed as bootstrapped correlation coefficient, along the study period 1954-2003. Lowercase and uppercase letters correspond to the months of the previous and current growth year, respectively

addition, phenological observations performed during 2014 showed a clear advancement at the two lowest sites (MER and VIP), as compared to a higher altitude (Souto-Herrero and García-González, pers. observ.). However, we found nearly no phenological displacement between MER and VIP, and therefore the more benign conditions alone do not appear to be enough to explain the big differences observed.

Differences in precipitation are more complex, because precipitation has a more variable pattern due to the topographic characteristics of the study area (Martínez Cortizas et al. 1999b). As opposed to temperature, a precipitation gradient is only observed at the three eastern sites, because regime during most of the rainy season is dominated by western and southwestern winds (Martínez Cortizas et al. 1999b), and they are located at leeward of the northern Galician Mountains (ca. 1,000 m asl.), whereas MER lies at windward. However, this would explain the differences between both lowest sites MER and VIP, but precipitation increases again because of elevation at EST and CAB, which are located within a much higher mountain range, so that we do not expect a strong influence of the Northern Galician

Mountains by blocking western oceanic air masses. In fact, MER and EST have a similar regime as regards annual precipitation and water balance (Table 2). On the other hand, late spring and summer precipitation regime is dominated by northern circulation, i.e., humid winds create cooler conditions than other areas in the region, which causes an environmental resemblance between MER and VIP. This is probably why differences between both sites were much less remarkable than for the earlywood variables, and the ordination for LW resulted to be much closer to a proper gradient.

These climatic differences, though remarkable, do not appear to be enough to explain the completely different behavior of MER, far from being integrated within an altitudinal gradient, especially at the early growing season. However, MER is not only the lowest site (higher temperature, less frost risk), but it also bears very important differences with regard to the soil type; and this fact should not be ruled out as a feasible explanation of such a different behavior, despite being a factor that does not show a year-to-year variation. MER is located on ultramaphic rocks (mainly peridotites that underwent further metamorphism), composed minerals such as olivine, pyroxene, and amphibole, with a very low content in silica; these rocks are easily weathered, resulting in soils with a higher pH value (5.5-6), and a different composition in mineral nutrients, as compared to the other sites. In contrast, the three other stands grow on acid soils (pH 4.5-5), specifically the geologic material is a mixture of acid schists (with a similar composition at all of them), and sandstone or quartzite (soil data obtained from Rodríguez-Lado et al. (2015)).

Floristic composition at the sites is also a clear indicator of the different conditions at MER. According to Izco et al. (1990), some of the low-elevation northern Galician oak forest present some elements that resemble 'subtropical' characteristics, due to the moist and warm summer. This includes 'laurel-type' trees as *Laurus nobilis* or *Arbutus unedo*, and a considerable abundance of ferns, including typical Macaronesian species (e.g. *Woodwardia radicans*, *Davallia canariensis*…), which were all present at MER, and not at the other sampled stands. In fact, Rodríguez Guitián (2010) includes this site as belonging to *Blechno spicantis-Quercetum roboris* subas. *lauretosum nobilis*, the most thermophile and moist facies of this kind of oak forests in the region.

Differences at MER with regard to the other sites were also observed in the overall distribution of vessels. In fact, vessel size clearly increased with elevation from VIP to CAB, regardless of considering the first row or the rest of the vessels. But MER did not fit within this gradient, with values similar to EST, much higher than expected for its altitude.

Chronology quality, variable selection, and climate-growth relationships

The analysis of earlywood anatomy and latewood chronologies, and their climate-growth relationships along an altitudinal gradient in NW Spain resulted in a complex pattern, without the identification of a clear factor constraining growth. However, this does not prove that conditions are optimal, as the climatic variables controlling growth might not be the same every year as a consequence of the oceanic climate, and therefore more difficult to identify than in the case of a prevailing limiting factor.

All evidences indicating that MER is a more favorable site for tree growth were also pointed out by the values of chronology quality. Year-to-year variability, expressed as mean sensitivity, was considerably lower for MER, showing the minimum values for all study variables in both earlywood and latewood. Similarly, common signal was also the lowest, and in general considerably lower as compared to the other site. Therefore, tree rings at MER should be considered 'complacent', i.e., tree rings with low both year-to-year variation and common signal, as a result of growing under favorable conditions (Fritts 1976). In fact, MER was the largest woodland in the area, and apparently well-preserved, with no evident signs of disturbance. Apart from this site, there was no other pattern of variation that could be related to the altitudinal gradient, except the mean

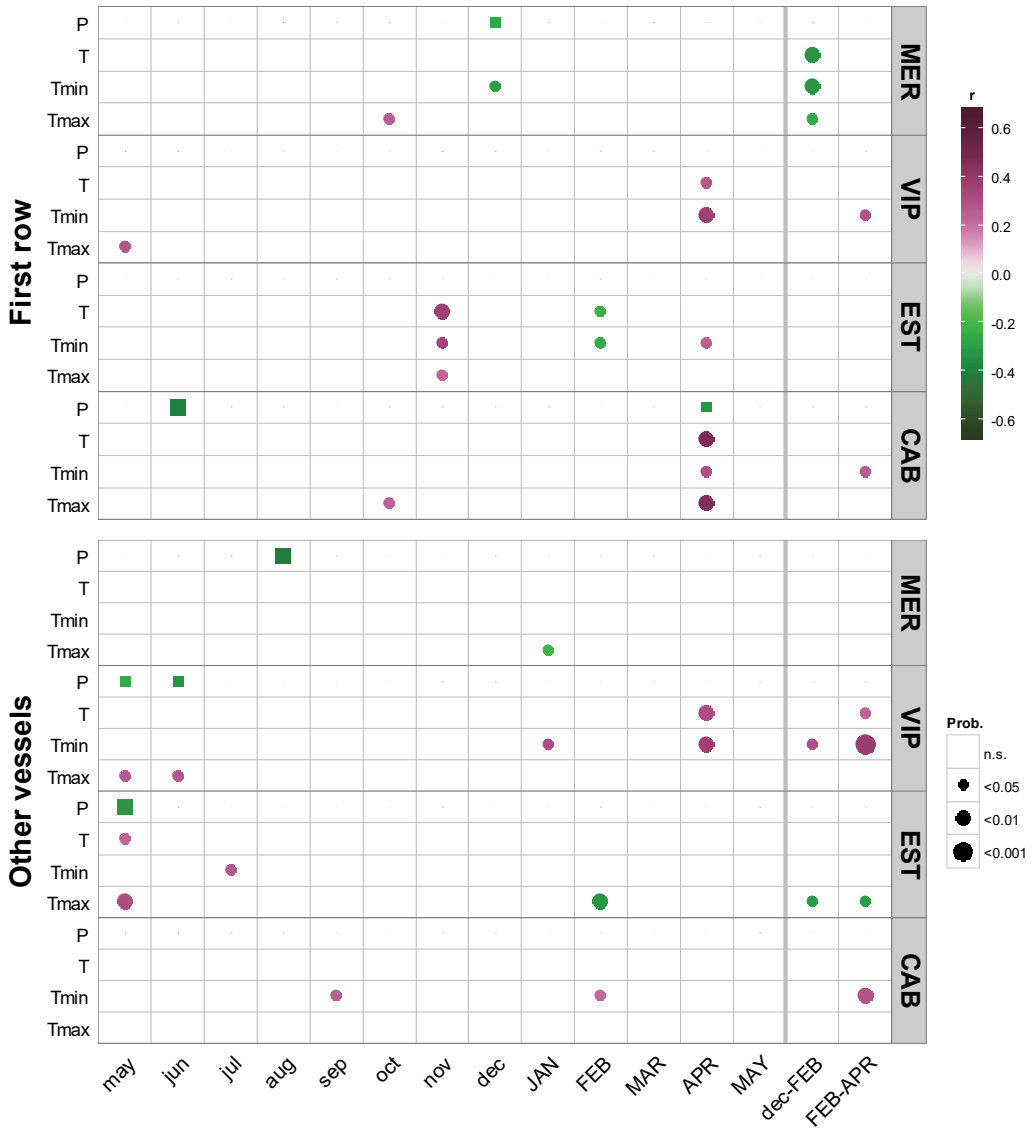

Fig. 7. Relationships to climate for hydraulic diameter (HYD), expressed as bootstrapped correlation coefficient, along the study period 1954-2003; vessel rows were considered separately. Lowercase and uppercase letters correspond to the months of the previous and current growth year, respectively.

sensitivity of LW, which clearly increased along with elevation, probably because of the more limiting conditions as the growing season is reduced.

Values of common signal were within the range observed in previous works for northern Spain, for both earlywood (García-González and Eckstein 2003; González-González et al. 2015) and latewood (Rozas and García-González 2012), or even higher in many cases. In general, earlywood anatomical variables have a very low common signal (Fonti and García-González 2008; Fonti et al. 2007; Gea-Izquierdo et al. 2012; González-González et al. 2014); however, many of our values were higher than usual, except for MER.

Climate-growth relationships were very complex for the earlywood, because they involved a considerable number of variables, and they often showed only weak responses. Vessel size, usual expressed as MVA, is the selected anatomical variable to understand the role of climate on earlywood formation in most cases (Abrantes et al. 2013; Campelo et al. 2010; Fonti et al. 2007; García-González and Eckstein 2003; Gea-Izquierdo et al. 2012; González-

González et al. 2014; Matisons et al. 2012). However, some authors were occasionally successful at using other proxies, as they appeared to record better the signals of spring conditions (Gea-Izquierdo et al. 2012; González-González et al. 2015), despite this is not the common situation. One of the most employed methods to reduce the number of variables for subsequent analyses is PCA (Bryukhanova and Fonti 2013; Fonti and García-González 2004; García-González and Eckstein 2003; González-González et al. 2015; Kniesel et al. 2015), but in some cases this has been done a posteriori, according to the relationships to climate (González-González et al. 2014), or even by filtering out an amount of vessels to optimize a specific signal (García-González and Fonti 2006). In our case, we used and intermediate approach, as we reduced the number of variables by PCA, while still maintaining a relatively high number of them (six in total), in order to improve our understanding on how climate affected earlywood formation.

The relationships among variables resembled those previously found, mainly separating vessel size and number (Fonti and García-González 2004; Kniesel et al. 2015). If using separated rows, González-González et al. (2014) showed that most size variables tended to group with the first row, whereas those not in the first row were more related to vessel number, with some 'intermediate' variables determined by both size and number. In our data, this was more a trend than a clear separation, except for CAB. This was probably due to the existence of numerous rings having an abrupt transition from earlywood to latewood, with few small vessels at the end of the earlywood. Variables not clearly splitting into groups at the other sites evidence the lack of a clear separation between the first vessel and those formed later, at least with regard to their behavior. Apparently, the longer and more active season at the lower sites could explain this result, as what we consider 'first row vessels', i.e., those formed at the beginning of spring, before bud break, and using reserves previously accumulated (Barbaroux and Bréda 2002) may span more than a row. But we cannot assure if the result at CAB is caused by more limitation because of the mountain environment, or linked to the fact that there were many more old trees, consequently with narrow rings.

Variables more determined by vessel number (TVA and NV) were studied together. But we found big differences between NV and the other variables, i.e., it was much more similar to ring width than to many earlywood traits. This was especially relevant at MER, the only site were NV was strongly related to climate. Specifically, a humid May favored the formation of more vessels, which should result an increment in radial growth; LW presented the same relation in June, confirming the association between both traits. In addition, both low elevation sites (MER and VIP) allowed identifying that earlywood formation was also dependent on previous year growth, as previously observed (González-González et al. 2014). No other remarkable relationship was found for NV, nor a pattern that could be related to the altitudinal gradient. A similar variable, TVA was even more unsuccessful at explaining the formation of earlywood along the gradient.

MVA and KS appeared to record a different signal. But unlike many previous works that consider this variable as the most appropriate one to relate to climate (Fonti and García-González 2004; Fonti et al. 2007; García-González and Eckstein 2003), we found some relations during spring, especially at high elevation, that should be considered very weak. In contrast, KS, the specific conductivity measured according to the Hagen-Pouseuille law (Sperry et al. 1994), has been seldom used when analyzing the earlywood vessels (Gea-Izquierdo et al. 2012); some previous works just considered the forth power of the radius, without weighting by the total conductive area (Fonti and García-González 2004; González-González et al. 2014). But in view of the results, we suggest that this variable should be further explored in the future, as it is directly linked to hydraulic properties (Gea-Izquierdo et al. 2012). In our case, it was capable of identifying opposite responses at the two extremes of the whole altitudinal gradient, namely a negative relation to spring temperature at MER, and

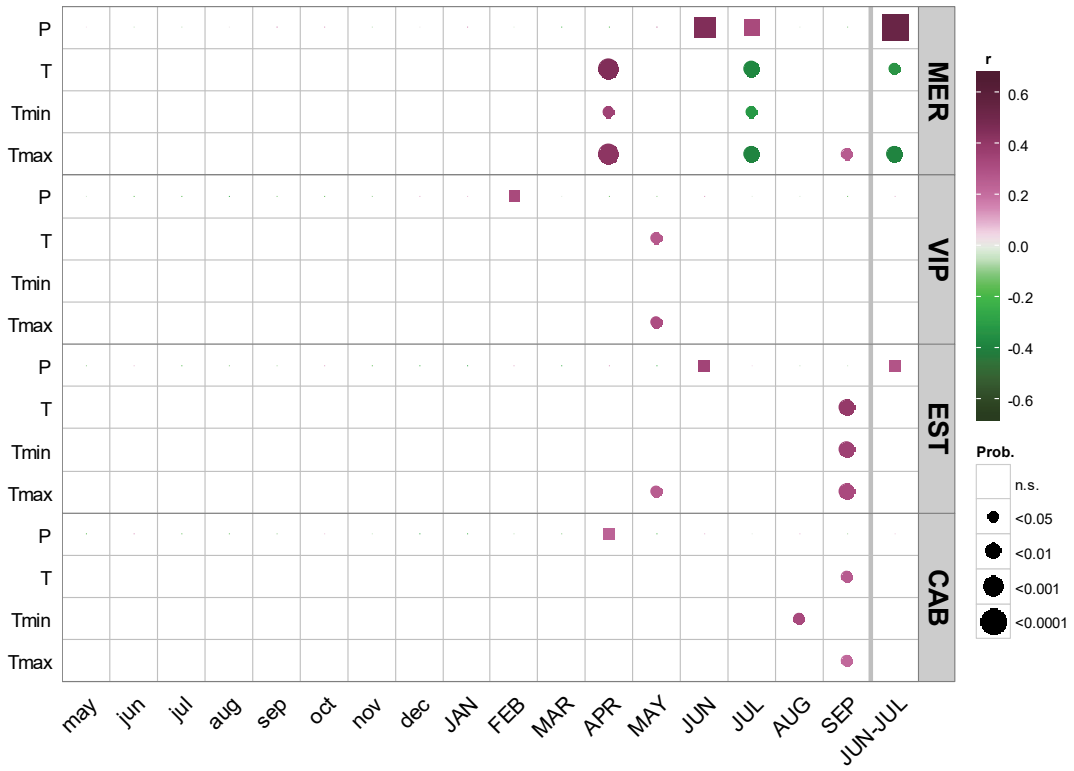

Fig. 8. Relationships to climate for latewood width (LW), expressed as bootstrapped correlation coefficient, along the study period 1954-2003. Lowercase and uppercase letters correspond to the months of the previous and current growth year, respectively

positive at CAB, with no response at mid elevation. The effect of late spring precipitation on NV at low altitude was also recorded.

We also studied vessel size (as expressed by HYD) as separated by rows, which provided the strongest relationships among all earlywood variables, especially for the first row. There was a negative relation to temperature in the dormant season only at MER, apparently related to processes of reserve consumption during the very winter at this site, as previously described by González-González et al. (2014). In contrast, April temperature favored vessel size at the other three sites, but with an elevation pattern. Whereas the correlation was maximized by the second row at VIP, it was the first row that showed this result at CAB. We hypothesize that this response is fired by a temperature threshold in spring; this would initiate the process of wood formation in the first row at the highest site CAB, while it would determine the rates of vessel differentiation at VIP; in addition, the response was very weak at the intermediate site EST, and absent at MER. Not only April, but earlier temperatures are probably acting at a lower elevations, because it was the period February-April that maximized vessel size for the second row at VIP. Temperature thresholds are probably not limiting at MER, so that and growth reactivation would only require that a certain photoperiod is accomplished (Basler and Koerner 2014). This explanation is compatible with our own phenological observations, and also with results obtained by Pérez-de-Lis et al. (2015), who found remarkable differences in spring phenology and wood formation along a gradient for *Q. robur* in NW Spain. In addition, earlywood vessel size of *Q. robur* resulted to be dependent on the duration of the enlargement phase, whereby an advancement of the growing season produced larger vessels (Pérez-de-Lis et al. 2015).

Climate-growth relationships for LW where rather unrelated to earlywood, except for MER, where a high precipitation during late spring-early summer favored both NV and LW. This result is similar to previous works, which found that water availability during summer was

related to radial increment of oak at lowland sites in northern Spain (García-González and Eckstein 2003; Rozas 2005). This was the only site in which radial increment was clearly facilitated by a higher water balance during summer. In addition, a high temperature in spring (April) was also positive for LW, probably by advancing the growing season, which indicates the high difference imposed by elevation. Whereas April temperature favored latewood growth at MER, it appeared to control the initiation of earlywood formation at CAB.

On the contrary, the other sites showed very weak associations between climate and LW, with only a weak positive effect of temperature and occasionally precipitation in spring, or at the end of summer. However, a strong year-to-year variability and a high common signal, which apparently indicate a stronger reaction to climate, are in contrast with the poor results for climate-growth relationships. This is probably indicating the difficulty of identifying the effect of climate on growth under the lack of a single prevailing limiting factor.

Conclusions

In this study, performed along an altitudinal gradient of *Q. robur* we found that climate growth relationships of oak are complex in oceanic areas, even close to the limit of its distribution boundary. Our gradient covered nearly the whole altitudinal range of the species in the area, but we did not find a clear driving factor that could explain the differences along with elevation. In fact, the lowest site was clearly discriminated from the others, due to milder climate conditions, and probably also to soil characteristics, which was also coupled to considerably differences in climate-growth relationships. Floristic composition at this site had was also very distinct from the others.

The separate study of earlywood anatomy and latewood increment was necessary to understand the effects of climate on growth, because both tree-ring compartments presented a completely different response to climate, except for the lowest elevation. The understanding of earlywood formation required the analysis of multiple variables, among which vessel size separated by rows was the most relevant. The responses were weak or moderate, and earlywood was usually related to conditions during its formation, either indicating cambial resumption or during vessel enlargment. A certain effect of the previous season, or during dormancy, were only observed at the lowest sites. Latewood was only clearly related to climate at the lower altitude, where it was controlled by water availability during summer.

In our opinion, the analysis and interpretation of climate-growth relationships of oak in an area where a prevaling limiting factor is lacking presents considerable difficulties. We consider that new and detailed information on phenology, wood formation, and ecophysiology are needed to ellucidate what climatic factors mostly affect tree performance.

Acknowledgements

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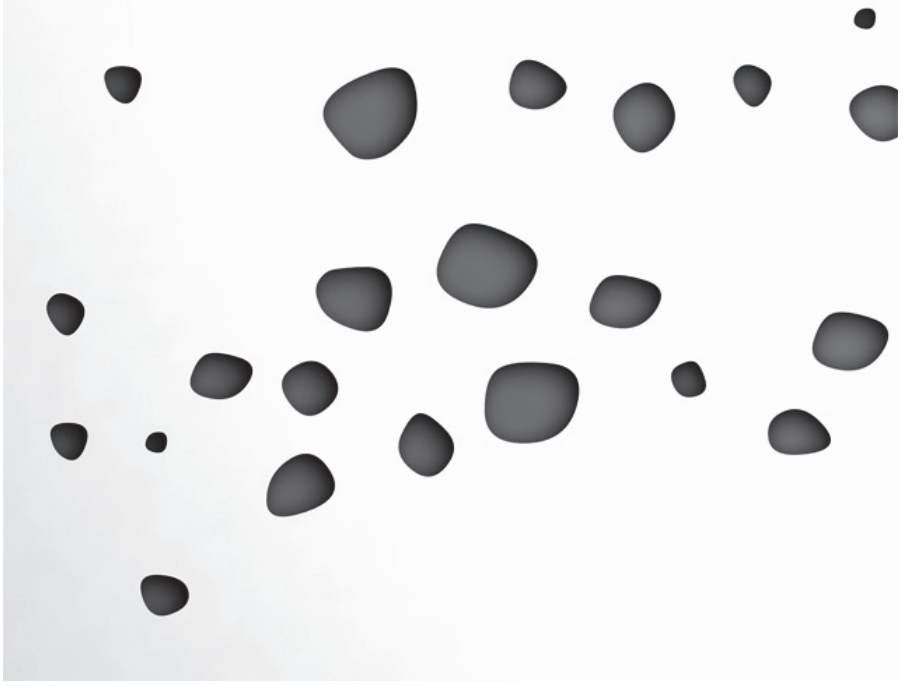
ARTICLE IV. EARLYWOOD VESSELS AND LATEWOOD WIDTH EXPLAIN THE ROLE OF CLIMATE ON WOOD FORMATION OF* QUERCUS PYRENAICA *WILLD. ACROSS THE ATLANTIC-MEDITERRANEAN BOUNDARY IN NW IBERIA



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Abstract

The deciduous oak *Quercus pyrenaica* is one of the most widespread species in pure or mixed forests across the Iberian Peninsula, and it mostly occurs in mountain areas within the Mediterranean region. It is a transition species, which dominates forests along the boundary to the Atlantic region in northern Spain. For this reason, it is important to understand the behavior of this species in a context of climate change.

We analyzed five stands dominated by *Q. pyrenaica* in northwestern Spain, which were located along a transect of increasing elevation along the boundary between the Atlantic and Mediterranean biogeographical regions. At each site, we sampled a representative number of trees, and obtained tree-ring chronologies by measuring the size of the earlywood vessels, and the radial increment. We selected three variables for further analysis, including the hydraulically-weighted diameter of the earlywood vessels, for the first row (HYD-r1) and for vessels not in the first row (HYD-nr1), and the latewood width (LW). Variable chronologies were compared to monthly meteorological records, and to the North Atlantic Oscillation index (NAO).

The results were considerable influenced by forest disturbance regime and by topography, more than by the altitudinal gradient. LW appeared to be controlled by water availability during late spring-early summer all throughout the study area, and the differences among sites were mainly determined by the presence of abrupt growth changes at several sites. On the contrary, the response of earlywood was more influenced by the topographic position of the sites. The three low elevation sites, located at windward of a central mountain range, seemed to be related to conditions during quiescence, whereas the two other sites responded at the moment of wood formation; HYD-r1 was much more related to climate than HYD-nr1. The association between NAO and vessel size was strong, and it could be explained attending at the elevation gradient.

Our results showed that *Q. pyrenaica* has a strong response to climate at micro-, meso-, and macroclimatic scales, which can be studied using both wood anatomy and radial increment, and it appears to be a suitable species to understand the behavior of tree species along boundary areas.

Keywords: Dendrochronology, tree ring, Oak, quantitative wood anatomy, climate-growth relationships

Introduction

Quercus pyrenaica is a winter deciduous oak that constitutes one of the most widespread tree species in the Iberian Peninsula. Its natural distribution area includes the Iberian Peninsula, southwestern France, and northern Morocco (Amaral Franco 1990); and it mainly occurs on siliceous soils within mountain areas in western Iberia (Sánchez-de-Dios et al. 2009). Areas dominated by *Q. pyrenaica* usually have a subhumid to humid precipitation regime, but this oak still supports a considerable water summer deficit (Hernández-Santana et al. 2008). It is considered the most abundant deciduous tree under continental Mediterranean climate (Hernández-Santana et al. 2009), and it usually forms monospecific stands, which has been traditionally managed as coppice or sylvopastoral systems (Gea-Izquierdo and Cañellas 2014).

One of the main adaptations of *Q. pyrenaica* is that it has a short growing season, which can span for no more than six months, because it is well-adapted to avoid late frost, and therefore most of the early phenological events are delayed. It is in fact the length of the growing season that can limit its geographic distribution area (Rico et al. 1996).

This species is considered to belong to the so-called 'marcescent' oaks, i.e., deciduous oaks that maintain their dry leaves on the tree for most of the winter, which constitutes an ecological adaptation to the special conditions of sub-Mediterranean (Sánchez-de-Dios et al. 2009). These are transitional territories between

the Eurosiberian and Mediterranean vegetation (Rivas-Martínez et al. 2001), where different marcescent oak species can occur, such as *Quercus faginea*, *Quercus pubescens* Willd. (= *Q. humilis* Miller), or *Q. canariensis* Willd., among which *Q. pyrenaica* is the most abundant (Amaral Franco 1990). But due to its transitional position, it is one of the species whose distribution area could be considerably modified under a climate change scenario as it could replace other temperate oaks if summer drought increases (Sánchez-de-Dios et al. 2009).

Understanding the ecophysiological requirements of *Q. pyrenaica* is essential to understand how Iberian forest can behave in the future, as it could affect the distribution of other tree species. However, it has been poorly studied (Hernández-Santana et al. 2009; Rico et al. 1996) despite being the most common deciduous tree species in Spain. Some works carried out in this species involve, e.g., stomatal responses (Rico et al. 1996), water use efficiency (Hernández-Santana et al. 2009), predictions models for future distributions (Sánchez-de-Dios et al. 2009), vegetation and floristic studies (Díaz-Maroto et al. 2007; Rivas-Martínez et al. 2001), dendrochronological analyses (Gea-Izquierdo and Cañellas 2013), or its combination to quantitative wood anatomy (González-González et al. 2013; González-González et al. 2014).

One of the most used tools to study the potential effects of global change on tree performance is dendrochronology, because of the annual resolution of the tree rings. In addition, techniques for tree-ring analysis have been extended in recent years with the use of quantitative anatomical parameters measured annually across sequences of tree rings (Fonti et al. 2010). Among these, one of the most promising parameters is the size of the earlywood vessels of ring-porous trees, like deciduous *Quercus*, which proved to have a strong and reliable relationship to climate (Fonti et al. 2007; García-González and Eckstein 2003; Gea-Izquierdo and Cañellas 2013), even in areas where 'classic' dendrochronology does not perform well, as is the case for mesic regions (Fonti and García-González 2008). Furthermore, time series earlywood vessels of *Q. pyrenaica* were successfully used to study climatic response (González-González et al. 2014; González-González et al. 2015), as well as those of other sub-Mediterranean oaks such as *Q. canariensis* (Gea-Izquierdo et al. 2012) or *Q. faginea* (Corcuera et al. 2004).

Several works point out that *Q. pyrenaica* has ecophysiological strategies that are different from those of temperate oaks, which indicate that it is in general more drought stress-tolerant. As compared to *Q. petraea*, Pardos et al. (2004) showed that *Q. pyrenaica* seedlings had a much more efficient osmoregulation, and Rodríguez-Calcerrada et al. (2008) observed that it performed more efficiently in light environments. In a comparative study using vessel chronologies at a site in the southern Cantabrian Mountains, González-González et al. (2014) found that the earlywood vessels of *Q. pyrenaica* appeared to be very plastic in order to adapt to environmental conditions, and hypothesized that this should be an advantage for this species to cope with climate change, as compared to other oaks like *Q. petraea*. Moreover, Pérez-de-Lis et al. (2015) confirmed the delayed phenology of *Q. pyrenaica* in regard to *Q. robur* along a gradient in northwestern Spain, and also found differences in wood formation dynamics, whereby *Q. pyrenaica* season length was shorter and unrelated to vessel size.

In this work, we use dendrochronology to analyze the earlywood vessels and radial increment of *Q. pyrenaica*, along a transect in the northwestern Iberian Peninsula. We study five stands on the boundary between the Atlantic and Mediterranean regions, which involve most of the altitudinal range of the species, as well as different climatic characteristics and disturbance regimes. Our main objective is to identify the most relevant environmental factors that influence wood formation across this transitional area.

Materials and Methods

Study area and sites

We sampled five *Quercus pyrenaica* stands at the northern distribution boundary of the species in

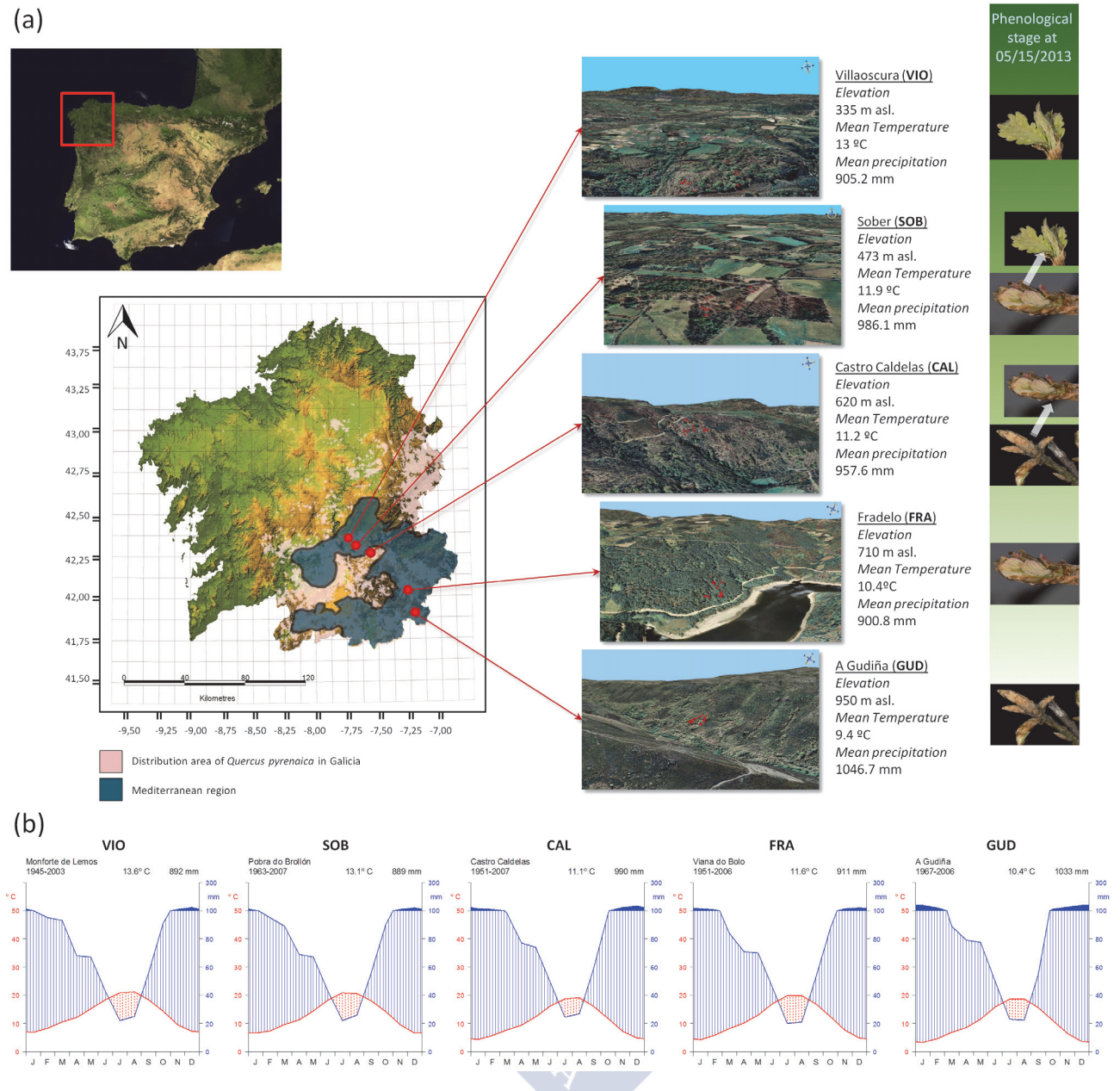

Fig.1. Location of the five chronology sites along the altitudinal transect (a), showing the climatic characterization and elevation of each one. Digital Terrain Model (DTM), with sampled trees as red points, and relevant phenological information for 2013 are shown, as well as corresponding climate diagram from nearby weather stations (b).

the Mediterranean region in Galicia (NW Spain). These study sites are located along an increasing elevation transect following a NW-E direction, within a mountain range between 42.04-42.48 ºN and 7.10-7.63 ºW (Fig.1), which covers the whole altitudinal range of this oak within the area. The stands represent a wide range of environmental conditions and disturbance regimes, so they should not be considered as a proper gradient. The lowest sites, VIO and SOB, are respectively located within and at the edge of a Tertiary basin, not exceeding 350 m asl., and mainly dominated by fluvial sedimentary materials. CAL lies on a northern slope of a mountain range facing the basin, and these three sites are separated from FRA and GUD by a granitic massif with peaks reaching 2000 m asl. (Fig. 2).

Climate at the study area is mostly dominated by an Atlantic precipitation regime, but with a remarkable seasonality which leads to

Mediterranean conditions during summer. Thus, rainfall is abundant (900-1,200 mm), and mainly concentrated in autumn and winter, but there is a remarkable summer drought, especially towards the lowland Tertiary basin, where temperature considerably raises. The inland location of the sites involves continentality, whereas altitude and orography are responsible for significant differences in rainfall and temperature patterns among stands (Table 1).

Sites are close to the boundary between the Eurosiberian and Mediterranean biogeographical regions, being all but CAL clearly located within the latter (Rivas-Martínez et al. 2001). All sampled woodlands but FRA fit into the association *Holco molli-Quercetum pyrenaicae* (Rivas-Martínez 1987), which are humid oak forests dominated by *Q. pyrenaica* occurring at the transition to the Mediterranean region. FRA corresponds to the subhumid *Genisto falcatae-Quercetum pyrenaicae* (Rivas-Martínez 1987), where more xeric characteristics should be expected. Stands at the lower distribution range of the species were traditionally coppiced in the past, or replaced by farmlands and recent conifer reforestations. As a result, the only forests available nowadays at these locations originate from abandonment, and are often very young.

VIO and SOB are both mixed woodlands of *Q. pyrenaica* and *Q. robur* on a gently slope facing south, and growing on a deep and fertile soil. Human pressure is notable in both cases, so that the understory includes species that belong to other plant communities, such as *Cytisus scoparius*, but also thermophile species as *Ruscus aculeatus*. VIO is a young and dense forest on a fluvial terrace, with few old trees on the deepest area. SOB is a small and sparse woodland with medium-sized trees, and old oaks on the edges, surrounded by younger oak forests from natural regeneration and meadows, on a soil prone to flooding.

The mid-elevation site CAL is located on a slope facing north-east, at the windward site of a mountain range, which causes a high local rainfall, and considerably moister conditions than at the other sites. Local microtopography facilitates the occurrence ofseasonal watercourses with abundance of *Q. robur*, and old *Castanea sativa* trees, silviculturally managed in the past, at the wettest areas. However, sampling was restricted to the less moist area of the hillside, a managed monospecific forest of medium-sized *Q. pyrenaica* trees on a deep soil, still accompanied by thermophile species as *Ruscus aculeatus* or *Genista falcata*.

Table 1. Climatic characterization of the study sites

Sites	VIO	SOB	CAL	FRA	GUD
Annual Mean Temperature (ºC)	13.0	11.9	11.2	10.4	9.4
Summer Mean Temperature (ºC)	20.0	18.6	18.6	17.3	16.0
Winter Mean Temperature (ºC)	7.2	5.8	6.0	4.7	3.4
Annual Mean precipitation (mm)	905.2	986.1	957.6	900.8	1046.7
Summer Mean precipitation (mm)	26.9	30.4	29.7	27.9	29.5
Annual Water Balance (mm)	266.0	391.2	292.2	317.0	465.3
Summer Water Balance (mm)	-83.8	-73.2	-77.0	-74.0	-68.4
Drought period and intensity	3 months/ High	3 months/ High	2 months/ Mild	2 months/ Moderate	2 months/ Mild
Climate description	Winters mild with late frost period. Summers warm.	Winters mild with late frost period. Summers warm.	Winters cold with frequent snowfalls and high rainfall. Summers temperate.	Winters cold, with frost period. Summers moderate warm and larger.	Winters long and very cold, with frost and snowfalls. Summers short and cool.

FRA is situated on a moderate east-facing slope, and close to a river reservoir, which increases the frequency of fogs. It is a monospecific woodland of *Q. pyrenaica* having a high tree density, with an abundant understory of *Prunus avium*, *Crataegus monogyna* and *Genista falcata*. A few old *Castanea sativa* trees at a small location, remains of an ancient chestnut plantation, are the only evidence of recent human activity, except some recent stumps resulting from occasional fuelwood extraction.

The highest site GUD faces north on a moderated slope of a narrow and elongated valley of glacial origin; despite the watercourse that runs at the valley bottom, there is no evidence of soil water accumulation. The woodland is monospecific, with medium-sized *Q. pyrenaica* trees, and a shrub understory of *Erica australis*. It is not managed at present, but logging and occasional fires affected this plot in the recent past.

Sampling, processing, and anatomical measurements

We sampled 15 to 20 dominant trees at each site (Table 2), by extracting at least two 5-mm increment cores at breast height. Cores were air-dried, glued onto wooden supports, and prepared in several steps in order to obtain an optimal visualization of tree rings and vessels. Samples were first cut using a WSL sliding microtome (Gärtner and Nievergelt 2010), until a regular cross-sectional surface was obtained; however, due to the hardness of wood, it was necessary to always follow this procedure by polishing, using progressively finer sandpaper (grain sizes from P220 to P1200, FEPA (Federation of European Producers of Abrasives) Abrasives) in order to completely remove knife spurs. Wood dust inside vessel lumina and tyloses were removed using high-pressure water blast (Fonti et al. 2002). Dry samples were finally stained with black printer ink, and earlywood vessels filled with chalk dust (González-González et al. 2014), achieving an optimal contrast for image analysis.

We measured earlywood width (EW) and latewood width (LW) to the nearest 0.001 mm in each tree ring, using a device integrated by a

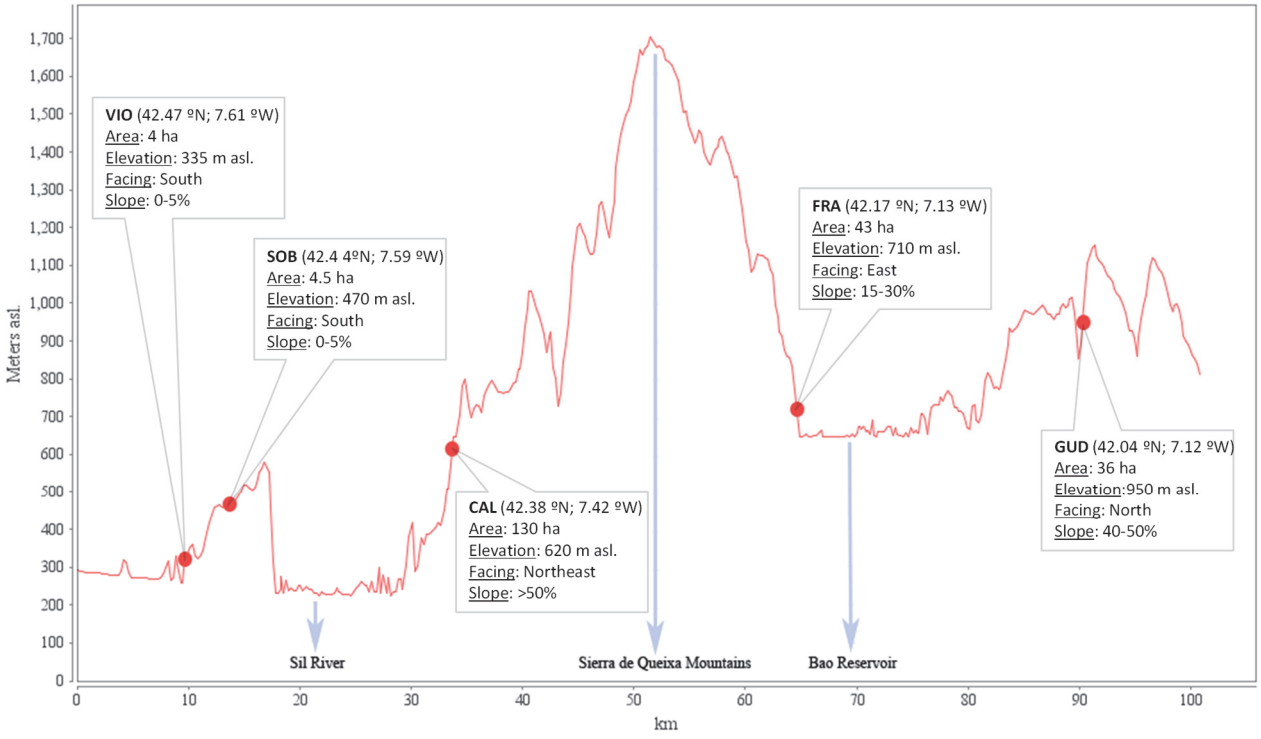

Fig.2. Northwest-East topographic profile, with physiographic characterization of the study sites.

tree-ring measuring linear stage (Velmex TA UniSlide, Velmex Inc., Bloomfield NY, USA) coupled to a binocular microscope (Olympus SZ60) at 20-40x magnification. The boundary between earlywood and latewood was set upon vessel size and wood structure (García-González and Eckstein 2003), as earlywood comprises a continuous band of considerably larger vessels, whereas smaller latewood vessels are disposed in flamelike groups along radial bands over a ground tissue of fibers. Crossdating was achieved by first comparing individual curves, and its accuracy was statistically verified using COFECHA (Grissino-Mayer 2001).

We selected a subset of 10-13 trees at each site in order to perform vessel measurements, keeping those with no breaks or anomalies, accurate dating to the local chronology, and enough age (Table 2). Cores were digitized using a device composed of a digital photo camera Canon EOS 600D, coupled to a binocular microscope Olympus SZ60, and a mechanically driven platform. We obtained sequential images at a high resolution (5184 x 3456, 17.9 Mpx) from surfaces of each wood sample. Pictures belonging to the same core were stitched (PTGui ver. 9.1.8 Pro, New House Internet Services B.V., Rotterdam, The Netherlands), and resulting images were saved into Tagged Image File Format (TIFF) files to avoid loss of quality.

Earlywood vessels were automatically measured in ImageJ (Abramoff et al. 2004; Schneider et al. 2012) by means of the VesselJ plugin (García-González, not published). We used an 8-bit (gray) threshold level, and vessel lumina (bright) were detected due to the contrast with the background tissue (dark). However, the correct recognition of all vessels was often hindered by the presence of dust spots or other undesired objects, which were detected as conductive elements. We applied therefore both a size and a shape filter in order to remove these objects, and consequently optimize the detection process. Minimum vessel size was fixed to 10,000 μm^2 (Eilmann et al. 2006; Fonti and García-González 2004), whereas circularity (minimum vs. maximum diameter) was set down to 0.5. But despite these procedures, manual corrections of the image to properly identify all conductive elements were still required in many cases. Vessel outlines were finally optimized by applying morphological operations (erode-dilate 2x2 one pass, and calculation of the convex hull).

Once all vessel lumina had been measured, we assigned them to the corresponding tree ring using the software Autovasos (García González, not published), specifically developed for ring-porous species. As this program also allows automatic tree-ring measurements based on vessel patterns, we checked the correct dating against previous curves in order to prevent the incorrect assignation of vessels. We split the vessels in each tree ring into two groups, by separating those belonging to first row, which usually bear a higher climatic signal (García-González and Fonti 2008), from formed later in the season. The first row included all vessels located immediately after the ring boundary up to those whose beginning did not exceed the imaginary line connecting the centers of the ring-boundary vessels.

Table 2. Number of trees used to build width and vessel chronologies, and for the characterization of the common interval at each site.

Site	Sampled/ Vessel trees	Time period covered	Common interval (c.i)	Trees covering c.i
VIO	18/10	1915-2012	1967-2012	8
SOB	15/10	1885-2012	1953-2012	8
CAL	20/12	1904-2012	1953-2012	10
FRA	19/12	1929-2012	1953-2012	10
GUD	20/13	1926-2012	1953-2012	9

Anatomical variables and analysis of chronologies

All measurements from both increment cores of the same tree were averaged into a single value for each ring, as variation within each individual was huge for anatomical parameters. Afterwards, we calculated numerous earlywood variables out of the initial dataset of vessel measurements, which were used for the subsequent time series. For all vessels, we considered the total number of vessels (NV); total vessel area (TVA); mean vessel area

(MVA); mean area of vessels larger than 90th percentile of each ring (M90); hydraulically weighted diameter (HYD = $\sum_1^n d_t^5 / \sum_1^n d_t^4$, for *n* vessels of diameter *d* in year *t*), which is proportional to xylem hydraulic conductivity (Fichot et al. 2009; Sperry et al. 1994); and the specific conductivity (KS) of the earlywood (kg m^{-1} s^{-1} MPa^{-1}) in year *t* according to the Hagen-Poiseuille law (Tyree and Zimmermann 2002), so that KS = $(\pi\varrho / 128\eta A)\sum_1^n d_t^4$, where ϱ is the density of water at 20 ºC (998.2 kg m^{-3}), η is the viscosity of water at 20 ºC (1.002 x 10^{-9} MPa), A is the area of the earlywood analyzed in yeat *t*, and d_t is the diameter of each of the *n* vessels measured in year *t*. NV, and TVA were standardized to a frame of 10 mm of tangential width, whereas the earlywood area to compute KS was estimated as the convex polygon including all vessels. In addition, we also considered size variables (MVA, M90, and HYD) for vessels belonging to first row (***r1***) or not (***nr1***) separately, because the selection of largest conductive elements at the beginning of the season usually improves the climatic signal (García-González and Fonti 2006; González-González et al. 2014). RW, EW, and LW were also included in the analyses, yielding a total set of 15 study variables.

Growth-related trends were removed from each individual series by adjusting a cubic smoothing spline (Cook et al. 1992) with a 32-years stiffness and 50% cutoff, which is flexible enough to minimize strong trends in the series, and suitable for different growth shapes that could appear in the different variables. Using a same detrending method ensures that differences among variables are not due to statistical procedures. Furthermore, the election of the spline stiffness should not affect the results, because trends in the anatomical series were in general smooth. We obtained the time series of growth indices by dividing each observed value by that estimated by the spline function (Fritts 1976), and finally averaged the indices into a chronology for each variable and site, using a biweight robust mean (Mosteller and Tukey 1977) to minimize the effects outliers. We did not prewhiten the resulting chronologies, as autocorrelation was negligible for most variables.

We achieved chronology quality according to standard dendrochronological procedures. This involved the computation of the mean sensitivity (*MS*), an indicator of interannual variability; the first order autocorrelation coefficient (*AR1*), which measures the influence of previous year on growth; the mean correlation between trees (*Rbt*), i.e., the mean value of all possible Pearson's cross-correlation coefficients, usually known as 'common signal'; and the expressed population signal (*EPS*), indicating the extent to which the sample size is representative of a theoretical population with an infinite number of individuals (Wigley et al. 1984). As many the variable chronologies were closely related to each other, we only kept the most relevant ones for the subsequent calculation of climate-growth relationships. Variable selection was carried out by principal component analysis (PCA), which proved to be very useful at identifying groups in time series of anatomical variables (Bryukhanova and Fonti 2013; González-González et al. 2014; Kniesel et al. 2015). PCA was applied on the correlation matrix of all variable chronologies at each site, and groups of variables yielding similar information were identified by loadings on the two first principal components following varimax rotation. The analysis was performed for the period 1967-2012 (42 years), since it was limited by the shorter chronology. According to the results provided by PCA, we finally selected three variables (MVA, HYD-r1, HYD-nr1) to characterize earlywood, and also LW to describe radial growth increment.

PCA analysis was also used to study the relationships between sites for each selected variable, applied on the correlation matrix of the five sites. So, we identified similarities or differences in the provided information, allowing us to know the degree of influence of elevation over each variable. In order to optimize comparisons, we performed all analyses for the period covered by at least eight trees (1953-2012, 60 years) at all sites, except for VIO, which covered a shorter period (1967-2012, 46 years) due to the presence of almost only young trees (Table 2).

As some of the sites evidenced recent management, we also tested if forest

disturbance regime could have some impact on the results. This was based on the frequency of abrupt growth changes (releases and suppressions) in the RW series. We followed the method of the percentage growth change, proposed by Nowacki and Abrams (1997), successfully adapted by Rozas (2005) for pollarded oaks in northern Spain. This method follows the equation $PGC=100*[(M_2-M_1)/M_1]$, where M1 and M2 are the means of the preceding and subsequent 10-year means when computing releases, and reversed if suppressions are calculated. We considered a release or suppression when fluctuations in growth rate were at least 50% in PGC as compared to the median, and the results were expressed as the proportions of trees showing an abrupt growth change per decade.

Climate-growth relationships

For each site, we compared variable chronologies to different climatic data sets for the period 1953-2012, and 1967-2012 for VIO, due to the shorter series length. Monthly series of temperature and precipitation for 1901-2013 were taken from CRU TS 3.22, a gridded source at 0.5º × 0.5º of geographical resolution available from the KNMI Climate Explorer (http://climexp.knmi.nl/). In addition, monthly indices of the North Atlantic Oscillation (NAO), used as an indicator of large-scale climatic variation, were directly obtained from the corresponding CRU database (http://www.cru.uea.ac.uk/cru/data/nao/) for the period 1821-2015, based on the Gibraltar observatory.

Climate-growth relationships were computed as correlation functions, i.e., we calculated the Pearson's correlation coefficient between the chronologies and each monthly meteorological value along the growing season. Significance level of the correlations was achieved by the bootstrap technique (Guiot 1991); we calculated 10,000 iterations for each coefficient, and applied the correction proposed by Mason and Mimmack (1992) for the percentile bootstrap confidence intervals. The season for climate-growth relationships comprised from previous May to current year May for earlywood, and to October for LW. We analyzed not only the relations for single months, but also for seasonal means integrating the periods for late dormancy and quiescence (February-April), and early spring (March-April, April-May, March-May) for earlywood variables; and current summer (May-July, June-July) for latewood width; we therefore established if the climatic signals were improved when analyze a longer time period.

Results

Statistical distribution of vessel size

We totally measured 169,101 earlywood vessels, with some variation across sites in their frequency (Fig.3). The highest number of measured vessels was recorded at southernmost site GUD, and the lowest for northernmost site VIO, but this number considerably depended on the number of rings measured.

Mean vessel area also differed among sites, with the maximum value at VIO and the lowest at CAL, whereas the three other sites registered similar values; the same pattern was observed for *r1*-vessels, and it was similar for *nr1*, with the only exception of the largest ones found at GUD. Vessels were considerably larger (71,753-57,938 μm^2 for *r1*) than for *nr1* (35,642-32,724 μm^2), with mean values of all vessels much closer to the smallest ones (49,284-42,967 μm^2). Therefore, *r1*-vessels were more scarce (36.2-43.1% of the total ring), and vessel distribution was consequently skewed to the smallest vessels. In fact, *nr1*-vessels had a clearly skewed distribution (skewness coefficient ranges 0.70-1.10), whereas *r1*-vessels tended to be nearly normally distributed (skewness ranging 0.00-0.39, being negligible for FRA and GUD). There was a certain spatial pattern across sites in the abundance of small vessels, as skewness progressively decreased from the northernmost site VIO to southernmost site GUD when observing *nr1*-vessels.

Comparison and selection of anatomical variables

The ordination of all 15 study variables on the space defined by the two first principal components tended to identify three groups of

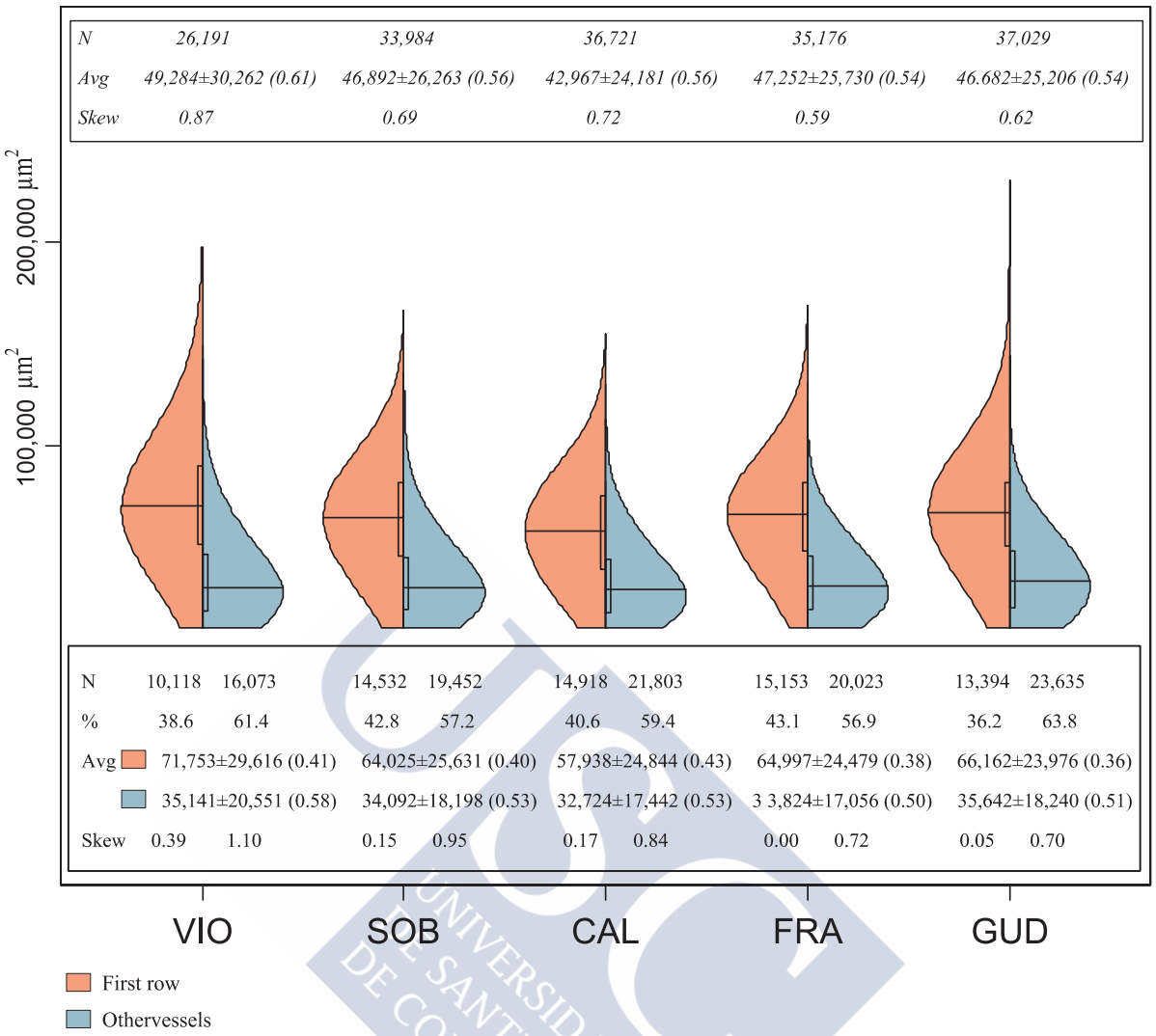

Fig.3. Violin plots showing vessel distribution at each site, with their corresponding descriptive statistics, for all measured vessels (italics, upper), and for the vessels separated by rows (lower). *N*: number of vessels; *Avg*: mean vessel area (µm²) ± standard deviation (coefficient of variation); *Skew*: Skewness.

variables, which apparently record a different kind of environmental information (Fig. 4). The result was similar for the five sites, despite some slight differences.

A first group was somehow describing cell production, as it included NV, RW, and LW, and this pattern was very consistent across sites. A second group comprised variables related to the size of *nr1*-vessels (MVA-nr1, M90-nr1, and HYD-nr1), whereas other variables affected by vessel number (namely TVA and EW) were somehow intermediate between the former group and this one, but considerably diverging among sites (e.g., clearly grouped to the *nr1*-vessels for FRA or VIO, but not at GUD).

A third, consistent group was integrated by variables related to vessel size for both all vessels and for *r1* (MVA, M90, HYD, MVA-r1, M90-r1, and HYD-r1), together with KS, which was considerably dependent on the largest vessels. This group was clearly discriminated from the others at all sites, except for CAL, where the transition from the *nr1* group was gradual.

These results were necessary for subsequent variable reduction, and consequently to facilitate

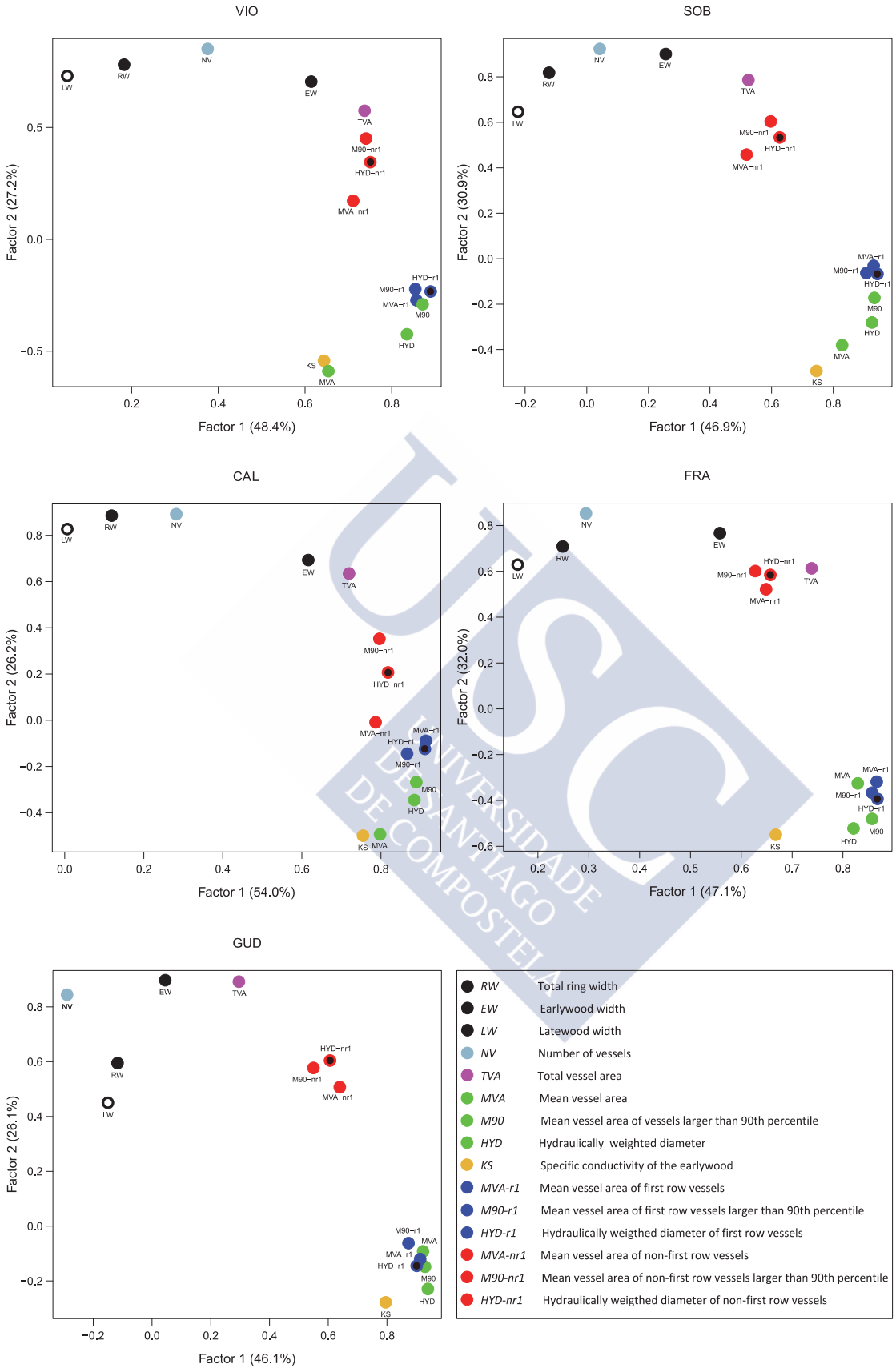

Fig.4. Principal component analysis (PCA) of the preliminary study variables along the period 1967-2012 for all chronologies, following varimax rotation. Double dots correspond to the selected variables.

further analyses. Thus, we selected four variables out the groups identified by PCA that were used for chronology assessment and climate-growth relationships. Radial increment was studied by using LW, i.e., the latewood portion of the ring, which is mainly due to the number of cell divisions along the season; we preferred LW to RW in order to keep the selected variable independent of earlywood values. As regards earlywood, PCA suggested that *r1* and *nr1* should be studied separately. We preferred to use HYD-r1 and HYD-nr1 for this, because HYD is more robust than MVA for being less affected by small vessels that do not contribute much to conductivity, even if both variables were highly correlated (r=0.95-0.98, P<0.0001 for r1, and r=0.88-0.93, P<0.0001 for nr1). Although vessel size in the first row was very similar to that of all vessels (in the case of MVA, r=0.65-0.80 for *r1* vs. r=0.48-0.74 for *nr1*), we decided to keep MVA for all vessels as an additional variable, because it somehow differed from HYD-r1 (r=0.59-0.75), and it is the most common climatic proxy used in previous anatomical works (Fonti and García-González 2008; García-González and Eckstein 2003; Gea-Izquierdo et al. 2012; Hroš and Vavrčík 2014), which would allow comparison of results. Correlations between HYD-r1 and HYD-nr1 were in general low, especially at a higher altitude (FRA and GUD), evidencing that the information contained in each one was rather different, and they deserve to be studied separately (Fig. 6).

Chronology quality for variables and sites

As a rule, LW, HYD, and MVA chronologies did not show a remarkable statistical quality. This was however much better for LW (Fig. 5), bearing an optimal common signal (Rbt=0.60; EPS=0.94) at FRA, and lower for the others, especially VIO and GUD, where LW chronology quality should be considered just acceptable. A main reason for this low common signal in LW is probably due to the presence of numerous abrupt variations (growth releases and suppresions) in many series, mainly concentrated at their beginning, but still present in the last decades. These growth variations are related to a strong human pressure, and were more common at low altitude (VIO and SOB), and in addition very recent at CAL. FRA showed a lower incidence of growth changes, which is probably the reason for its better common signal; as regards GUD, only the first years of the series appeared to be affected by such disturbances, probably related to recurrent wildfires. Year-to-year variability attained its lowest value at GUD (MS=0.21), and it was only slightly higher at the other sites (MS=0.26-0.27); as regards autocorrelation, this can be considered negligible for all sites after detrending, and confirms that a prewhitening of the series is was needed.

All earlywood variables clearly had a much lower common signal, but HYD-r1 (Rbt=0.13-0.26) had in general the best quality (Fig. 6); EPS was clearly below the values usually found for RW, and this is critical for MVA at VIO. The pattern among sites reflected that observed for LW, and FRA was undoubtfully the site showing the highest signal. Though low, there was still a certain year-to-year variability that deserves to be studied dendrochronologically, and all series shoud be considered to have no autocorrelation.

PCA analysis on sites indicated a different pattern depending on the variable considered (Fig.7). LW did not follow any ordination in accordance with the conditions across the transect, but it was completely driven by disturbances; the recently managed CAL clearly separated from the other sites, whereas FRA was located opposite to it. When both earlywood vessel rows were considered together, as is the case for MVA, the ordination appeared to follow elevation, but it was broken by the behavior of VIO, probably due to a much higher proportion of juvenile wood at this site. However, the importance of the elevational distribution was clearer for HYD-r1 and HYD-nr1, as sites tended to arrange along the altitudinal transect, although a perfect ordination was not achieved.

Climate growth-relationships

Correlations between LW and climate variables showed a certain similarity across sites, and revealed the role of early summer conditions, especially rainfall (Fig.8). However, the variation

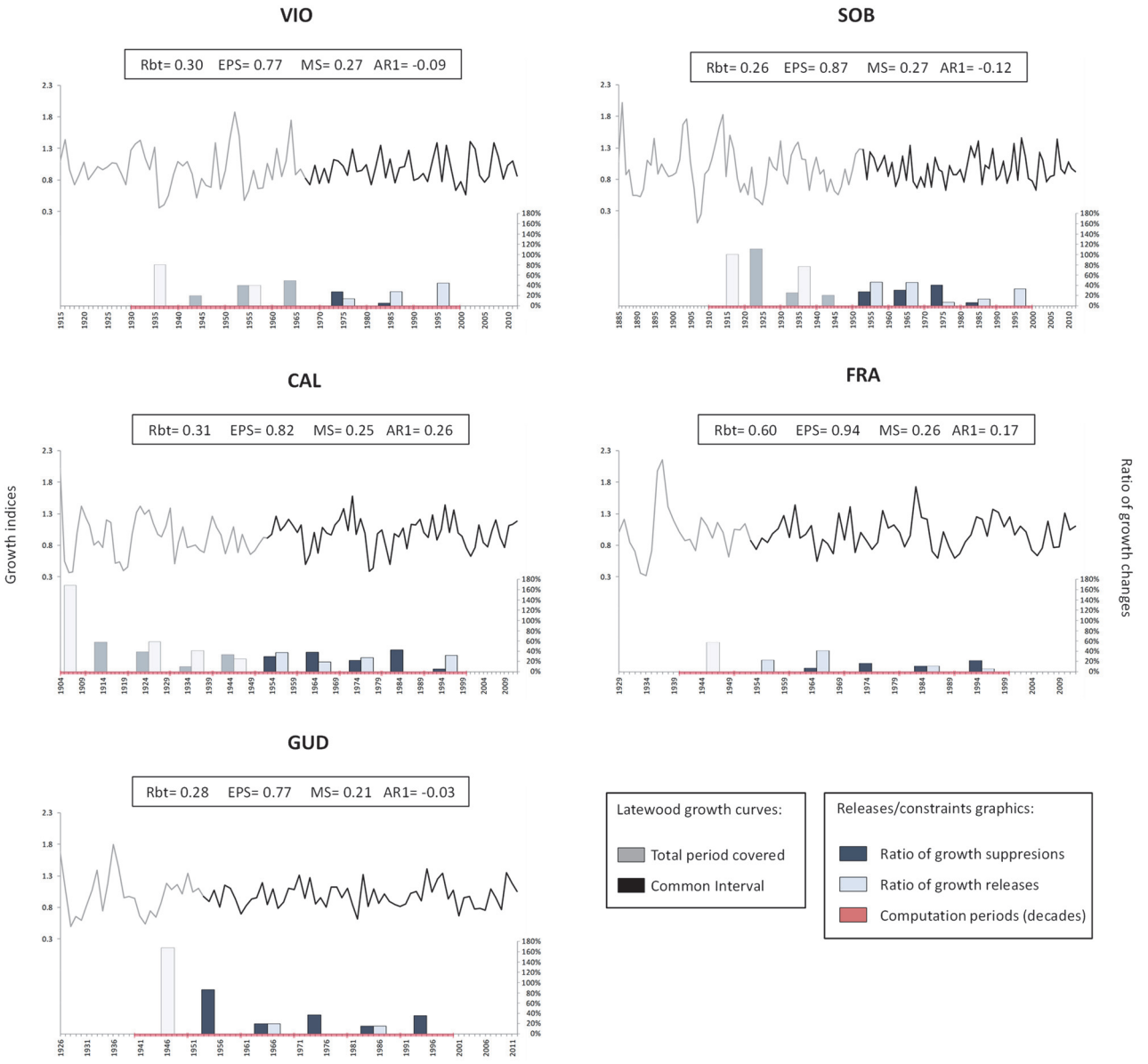

Fig. 5. LW chronologies, with descriptive statistics of chronology quality. Histograms represent the percentage of growth changes per decade. *Rbt*: mean correlation between trees; *EPS*: expressed population signal; *MS*: mean sensitivity; *AR1*: first order autocorrelation coefficient.

pattern observed in the strength of the responses does not appear to be related to any gradient, or even to the different weather conditions across the transect, but just linked to the common signal at each site. This is probably due to the masking effect of abrupt growth changes on climate. Thus, precipitation exhibited a significant positive correlation between May and July for the five plots, but it was clearly higher at FRA (r=0.57, P<0.0001), the most homogeneous chronology, while very low at VIO (r=0.21, P<0.05). Negative relations to early summer temperature were also highly significant at FRA (r=–0.41, P<0.001 for May-July); there seems to be a certain variation along with elevation, as the correlation was maximized in May at FRA, but recorded in June at GUD (r=–0.38, P<0.01). Responses were wery weak for the most disturbed stands, namely the mid-elevation site CAL, and the youngest VIO, although the relation to June precipitation was still present (r=0.23, P <0.05; r=0.33, P <0.05). SOB was also strongly correlated to June precipitation (r=0.36, P<0.0001), but also to conditions probably linked to cambial reactivation as shown by the minimum

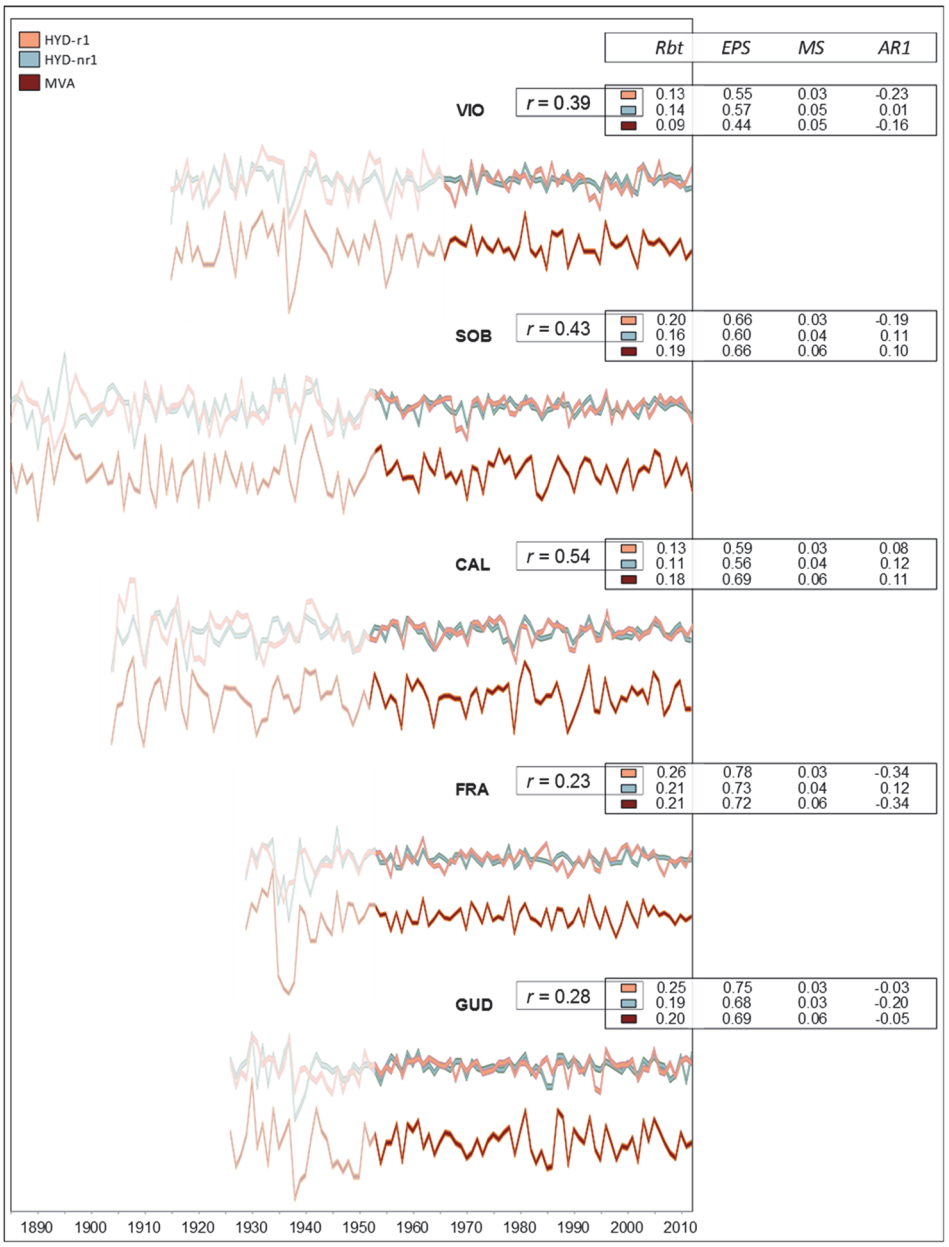

Fig.6. Chronologies of HYD (separated by rows) and MVA (all vessels), with their corresponding descriptive statistics for each site. *Rbt*: mean correlation between trees; *EPS*: expressed population signal; *MS*: mean sensitivity; *AR1*: first order autocorrelation coefficient. Dark lines represent the period for the analyses. Pearson's correlation coefficient (*r*) between vessel rows is given.

temperature in April (r=0.38, P <0.0001). A weak positive relation to the temperature during the dormant period was observed for all plots but VIO, though very weak at GUD. No strong relations were found between NAO and LW.

Climate-growth relationships of vessel size, as expressed by HYD, exhibited a much remarkable variation across sites, and completely differed between vessel rows (Fig. 9). Apart from several weak relations to conditions in the previous summer, most responses of HYD-r1 to climate were concentrated during late winter and early spring. All sites but CAL were positively linked to spring precipitation at a certain moment between March and May, although correlations were low(r=0.33, P <0.05 at SOB; r=0.29, P <0.05 at VIO), except for FRA, which recorded the highest value (r=0.37, P <0.0001 with

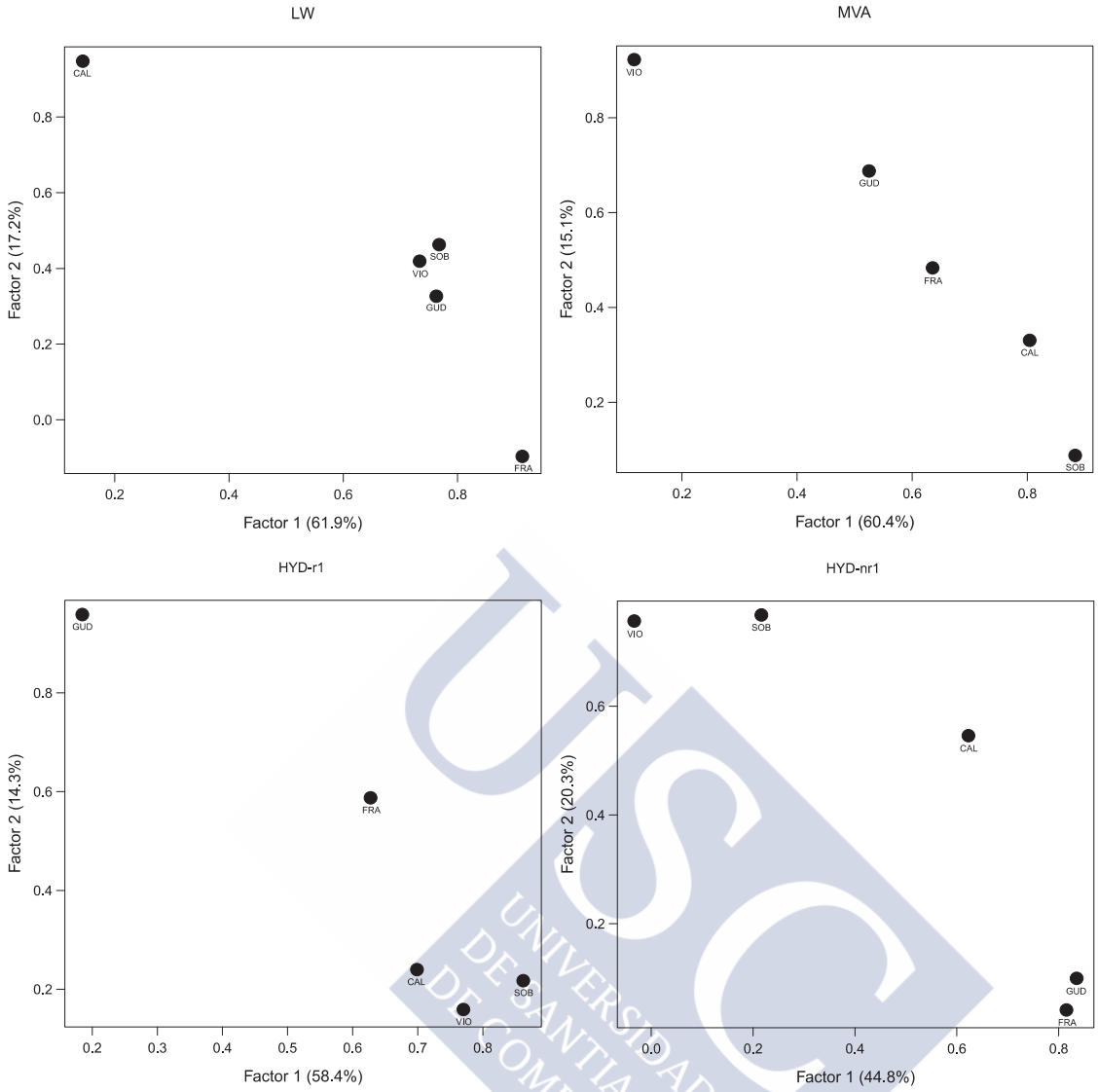

Fig.7. Principal component analysis showing the ordination of sites on the space defined by the two first eigenvectors, following varimax rotation along the periods 1953-2012 and 1967-2012 (only for VIO).

April); there was no time shift in the response to precipitation that varied along with elevation. On the other hand, correlations to temperature were generally stronger, with the exception of the low-elevation VIO. The role of temperature at the end of dormancy was clearly present at mid- elevation, as SOB and CAL showed a high correlation to temperature in February (r=–0.37, P <0.01; r=–0.41, P <0.0001 respectively), apparently optimized by the minimum temperature, but weak or absent to maximum temperature. This effect was weaker at FRA, where spring precipitation was maximized, whereas February temperature did not appear to play any role at both lower and high extremes of the altitudinal gradient. Furthermore, minimum temperature in spring had the opposite relation for the upper sites, with a positive effect on vessel diameter that was clearly maximized at GUD (r=0.46, P <0.0001). Relationships between climatic variables and HYD-nr1 were completely unrelated to those of the first row. In fact, these vessels appeared to be much more independent of climate, and only May temperature (mainly maximum) seemed to be inversely coupled to vessel size at FRA (r=–0.40, P<0.0001), i.e., a higher temperature resulted in smaller vessels probably by accelerating vessel differentiation. On the other hand, the amount of significant correlations found with splitted HYD contrasted with the poor results using MVA (Fig. S1), confirming that the study of separated rows is necessary to improve climate-growth relationships. The

effect of local climate on vessel formation is probably mediated by NAO, as it is highly related to winter and early spring conditions in the western Iberian Peninsula (Fig. S2). In fact, some remarkable correlations were found to NAO values during quiescence and vessel formation (Fig. 10), and these relations appeared to follow a clear altitudinal pattern. NAO in late winter (February-April) was related to HYD at the lowest sites VIO and SOB, which recorded this signal in the first row (r=–0.49, P <0.001; r=–0.40, P <0.001 respectively). Mid-elevation sites CAL and FRA registered the NAO signal later in the growing season (March-May), and almost exclusively on vessels not belonging to first row (r=–0.39, P <0.001; r=–0.57, P <0.0001). Finally, we found no significant correlation at the high-elevation stand GUD for any subset of vessels; furthermore, the influence of NAO on regional climate seems to disappear towards the end of spring (S.2).

Discussion

As a typical transition species between Atlantic and Mediterranean environments, *Q. pyrenaica* is widespread in the northwestern Iberian Peninsula (Díaz-Maroto and Vila-Lameiro 2007), mostly occurring in inland and mountain environments, either as monospecific stands, or mixed with *Q. robur*. But with the exception of a few warm coastal enclaves where *Q. pyrenaica* occurs locally close to sea level (Rozas et al. 2009), we were able to cover the whole altitudinal range of the species within the area. Due to the complex topography, a continuous alternation of mountain ranges and valleys that considerably modifies precipitation regimes (Martínez Cortizas et al. 1999), we cannot consider our study sites as an altitudinal gradient, but as a transect along the Atlantic-Mediterranean boundary (Rivas-Martínez 1987). Moreover, we aimed at representing the main environments where *Q. pyrenaica* is dominant, and our sampling design included variations in

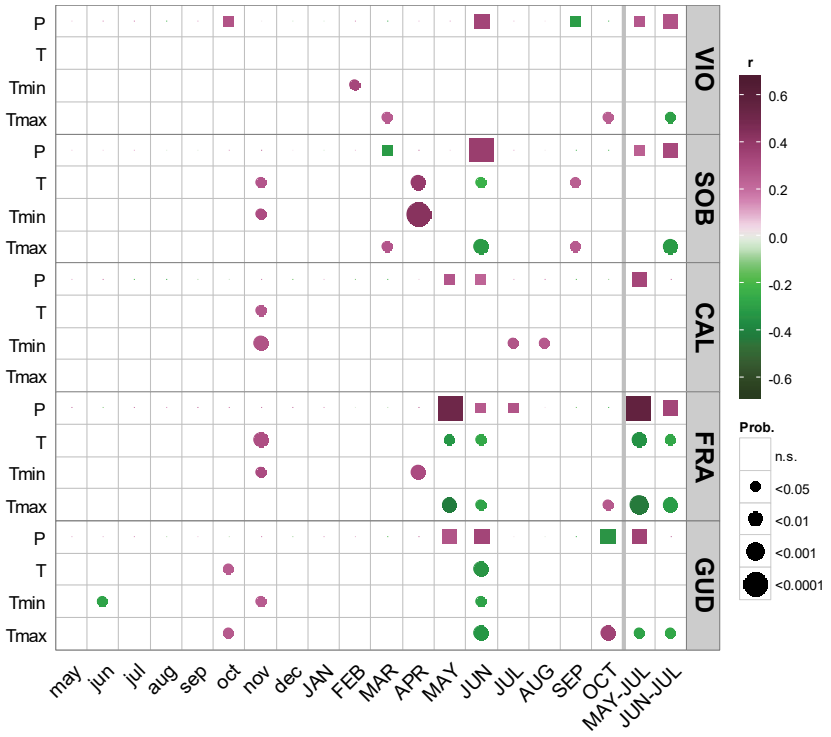

Fig.8. Relationships to climate for latewood width (LW), expressed as bootstrapped correlation coefficient, along the study periods 1953-2012, and 1967-2012 (only VIO). Lowercase and uppercase letters correspond to the months of the previous and current growth year, respectively.

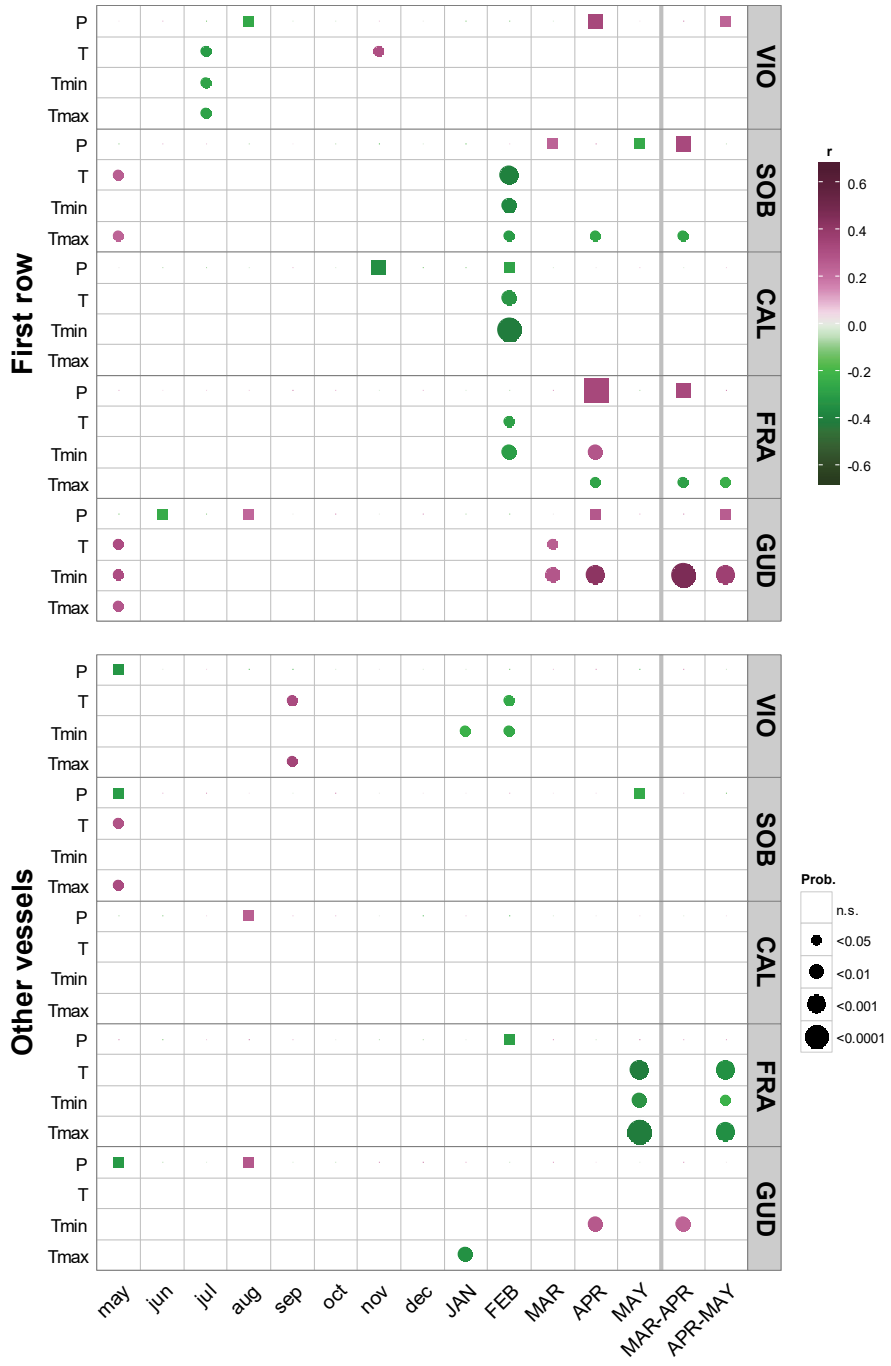

Fig.9. Relationships to climate for hydraulic diameter (HYD), expressed as bootstrapped correlation coefficient, along the study periods 1953-2012, and 1967-2012 (only VIO); vessel rows were considered separately. Lowercase and uppercase letters correspond to the months of the previous and current growth year, respectively.

altitude, local humid conditions, or different disturbance regimes, in order to better to understand the main effect of climate on this species. Consequently, most of our results cannot be interpreted as the sole consequence of elevation. Thus, the ordination of site chronologies showed this above-mentioned heterogeneity, since it was very different depending on the growth variable considered. In the case of LW, it was clearly influenced by disturbances; CAL, the site recording more recent growth changes, was completely separated from the other sites and opposite to FRA, the most homogeneus stand. On the contrary, HYD tended to organize according to topographic position of the sites.

The selection of variables was straightforward, since there was a clear separation at the five sites, evidencing that *Q. pyrenaica* is very homogeneous for the selected variables. The reason for this homogeneity as compared to *Q. robur* or *Q. petraea* is probably that *Q. pyrenaica* is a late flushing species, which results in a short growing season, with the mainly active period comprised between the end of frost risk and summer drought; in fact, Pérez-de-Lis et al. (2015) showed that the period for wood formation was much shorter for *Q. pyrenaica* than for *Q. robur*. It also confirms previous studies showing that vessels in the first row were related to vessel size, while the others to vessel number (González-González et al. 2014; Kniesel et al. 2015). For this reason, the selection of only vessel size variables based on rows, HYD in this case, appears to be appropriate. In addition, the close relationship between NV and LW (and consequently ring width) is an evidence that the growing season is short for this species, and that the formation of the last earlywood vessels and latewood is close in time, probably controlled by similar factors.

Climatic response for radial increment, as expressed by LW, can be considered robust, but there are many cases in which forest management clearly masked the signal, as was the case for CAL and VIO. In general, a high common signal was also coupled to a high climatic signal (SOB and FRA). The method employed to detect abrupt growth changes, adapted from Rozas (2006), was successful at identifying the reasons for the lower climatic signal at some sites. Therefore, according to our findings *Q. pyrenaica* is a species that does not exhibit a considerable variation in latewood formation within the area, since we studied the whole altitudinal range and did not find much differences in climate-growth relationships.

In general, we appreciated a positive effect of precipitation during late spring and early summer, and the opposite relationship for temperature, pointing out that it is water availability during this period that controls the formation of the latewood, and consequently most of the variation in ring width. This is supported by previous works, as Gea-Izquierdo and Cañellas (2014) found that cumulative

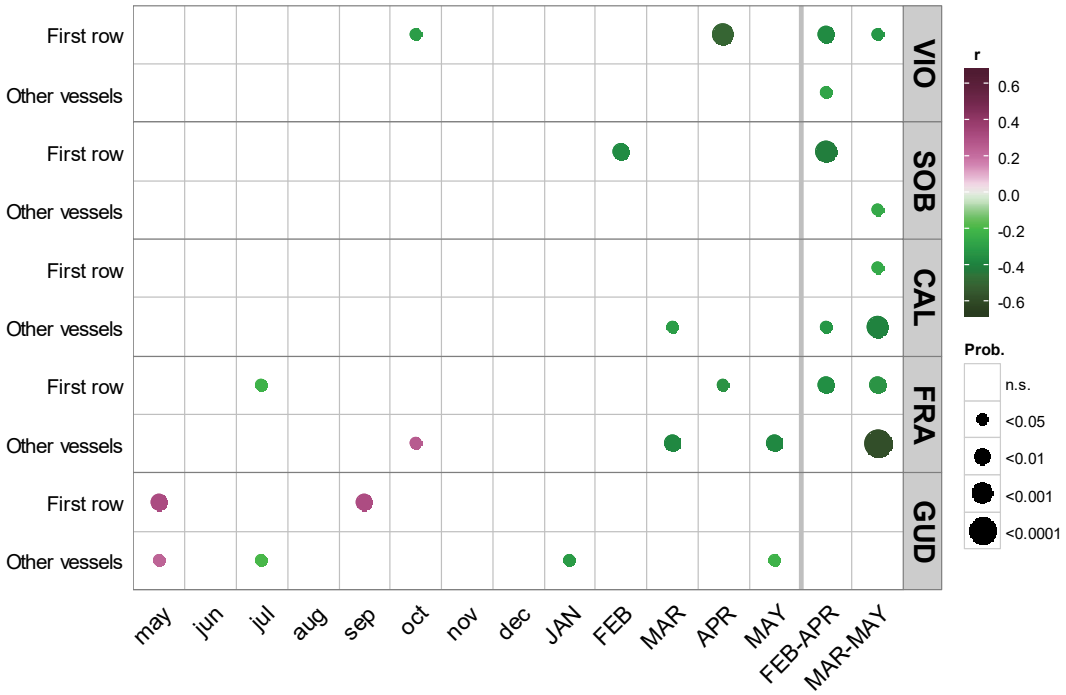

Fig.10. Relationships between NAO and the hydraulic diameter (HYD), expressed as bootstrapped correlation coefficient, along the study periods 1953-2012, and 1967-2012 (only VIO); vessel rows were considered separately. Lowercase and uppercase letters correspond to the months of the previous and current growth year, respectively.

precipitation was the main factor controlling growth of Q. *pyrenaica* along the Iberian Peninsula. In our case, relationships to water availability already begin in May, and extend until June and July, depending on the site. In fact, *Q. pyrenaica* occurs in Mediterranean areas, where summer drought is important (Sánchez-de-Dios et al. 2009), and therefore, it seems that the species does not carry out a considerable increment during the most important part of the summer. According to Pérez-de-Lis et al. (2015), earlywood formation of *Q. pyrenaica* in NW Spain can extend up to early July, so we do not think that the whole response found is concomitant with latewood formation, but affecting other processes beforehand, which in turn determine the increment on latewood. Favorable conditions could also anticipate the formation of latewood instead of earlywood. In addition, there appears to be a small growth pulse in October if weather permits. This somehow bimodal growth is typical of Mediterranean environments (Camarero et al. 2010; Campelo et al. 2009), and has also been appreciated for oaks in NW Spain, which has been observed studying cambial activity (Pérez-de-Lis, pers. com.). Though with a weak response, a high temperature in previous autumn also seems to stimulate LW growth in the following growing season, probably by prolonging the period for assimilation; this effect should be somehow related to the dynamics of reserves storage, but the understanding of such relationship is out of the scope of this paper.

Although we observed this general pattern of climate-growth relationships, there were important differences across the transect, which can be easily explained attending at site characteristics. The strongest response was found at FRA, situated at leeward of high-elevation massif known as Serra de Queixa, so that oceanic winds get drier, and therefore latewood growth more dependent on water availability. Despite being close to FRA, this was not the case for GUD, because the higher elevation increases precipitation and decreases evapotranspiration, resulting a lower limitation of water availability; in addition, the response was optimized by June instead of May, probably due to a delay in cambial activity, and also in the beginning of the drought period. Moreover, the low common signal and weakest relation to climate at GUD suggest that *Q. pyrenaica* is probably close to its optimum at this location. At low elevation, SOB exhibited also a strong climatic control by late spring precipitation, but it had in addition an effect of April temperature, probably related to anticipation or acceleration of phenological processes under favorable conditions. In contrast, VIO and CAL had a weak relation to water availability, though still present, but disturbance was important at these managed sites, therefore masking this effect; CAL has in addition a higher water availability, which should be also contributing to the observed response.

Whereas the climatic control of latewood presented a general pattern, which was slightly modified locally by site conditions, earlywood responses to climate (expressed as HYD) were in general site-specific. Most of them were related to the first vessel row, with only FRA having a strong response in other vessels. Besides, the effect of forest disturbances does not appear to be present as in LW, but there was either an important variation along with elevation, or an effect of the mountain range separating windward and leeward sites.

Previous works showed that earlywood vessels responded to climate in three key periods, i.e, in the prior season (González-González et al. 2014), during dormancy or quiescence (Fonti et al. 2007; González-González et al. 2015), or at the moment of their formation (Fonti and García-González 2008; García-González and Eckstein 2003). All three periods were found in our paper, though responses during the previous season were weak, and it was the end of dormancy that was mostly related to vessel size.

All three low elevation sites (VIO, SOB, and CAL), located at windward, were inversely related to temperature during February. The effect of climate was rather unclear at VIO, but this site mostly comprised young trees, and covered a shorter period than the others. In this case, the effect of February was better observed in MVA, probably because the presence of

earlywood in juvenile wood with multiple rows of small vessels (Gasson 1987; Helinska-Raczkowska 1994) does not provide good traits when considering rows separately. But the two other mid-elevation sites had a strong relationship to temperature during February, whereby a high temperature resulted in small vessels, and vice versa. At a similar elevation, Pérez-de-Lis et al. (2015) revealed that *Q. pyrenaica* initiated cambial activity showing divisions from late February to early April, with vessel enlargement from mid-March, and budburst coupled to the beginning of secondary cell wall formation. As our oaks registered the effect of temperature just before this activity, the role of temperature must be related to quiescence. Such a relationship at the end of winter has also been found for *Q. petraea* and *Q. pyrenaica* (González-González et al. 2014), or *Castanea sativa* (Fonti et al. 2007), who proposed an enhanced sensitivity in the cambium as a feasible explanation. During quiescence, cambial cells undergo structural (Farrar and Evert 1997) and hormonal (Lachaud et al. 1999) changes that predispose them to respond later in the season. Other responses of oak including this period were proposed to involve the possibility of carbohydrate consumption due to maintenance of respiration (González-González et al. 2015). The fact that the signal is lost in elevation sustains this hypothesis, but it would be more reliable if the signal extended from a longer period spanning most of the dormant season. In addition, Pérez-de-Lis et al. (2015) did not find any relationship between the initiation of vessel expansion and their final size for *Q. pyrenaica*, so that an advancement of cambial reactivation would be also insufficient to explain the role of temperature. Consequently, we hypothesize that the effect of temperature in February is probably predisposing trees to further responses later in the season. This only affects the initial vessels, as those not located in the first row did respond to this climatic factor.

At FRA, the lowest site on leeward, a weak effect of February was still present, but the main response was the positive effect of April rainfall on vessel size of the first row. Both our own phenological observations, and previous works on the timing of phenology and earlywood formation in the region (Pérez-de-Lis et al. 2015) suggest that cambium was already active in April, and therefore the control of precipitation should be direct upon vessel enlargement, as previously observed in other dendrochronological works (Fonti and García-González 2008; García-González and Eckstein 2003). Water availability controls growth probably due to turgor pressure on expanding cells (Abe et al. 2003; Woodruff et al. 2004), which could affect both vessels and opening buds, which occur in this period near the study area (Pérez-de-Lis et al. 2015). Vessels not in the first row were mostly negatively related to temperature later in the season, namely May; this effect should also be direct, since a high temperature should trigger a faster differentiation, accelerating secondary wall deposition, and resulting therefore in small vessels. Moreover, expanding leaves in May are a major source of auxin, the main hormone responsible for vessel differentiation (Sorce et al. 2013). This response, though weaker, was also observed for LW, but prolonging later in early summer; a high temperature coupled to a low precipitation would increase the synthesis of gibberellin and induce latewood formation following leaf maturation (Aloni 2015). Consequently, many of the vessels measured for these rows at FRA were probably in transition to latewood.

Finally, the different response at the high-elevation site GUD, a positive effect of March-May temperature, is probably linked to colder spring conditions. As no studies are available on the control of phenological events in *Q. pyrenaica*, we cannot rule out that the initiation of cambial activity is mainly controlled by photoperiod at a lower elevation, being unable to reactivate under early favorable conditions in order to prevent late frost. On the contrary, a temperature threshold would be reached later at a higher altitude, once photoperiod requirements had been met. This would indicate that the temperature signal that at GUD is probably related to cambial resumption.

We found two different levels of response to climate between earlywood and latewood. Whereas latewood seemed to describe a general

limitation of the species in the study area, earlywood appeared to be a better indicator at a local scale. This partly contradicts what was found by (Fonti and García-González 2004) for chestnut, who suggested that earlywood was probably recording a macroclimatic signal, and suggest the necessity of more studies to elucidate the importance of the different variables at both a regional and local scale. But despite earlywood was linked to micro- or mesoclimatic conditions, it was very efficient at identifying the effects of NAO, proving its usefulness for climate-related studies. Earlywood vessel size, expressed as HYD, was inversely related to NAO from February to May, i.e., the period probably including all processes of cambial reactivation and earlywood formation. But the most relevant result of this relationship is the clear gradient observed, as the lowest sites responded earlier (February-March), and this signal shifted towards later periods (March-May) in altitude, increased its intensity, and changed from the first row to the other vessels; the highest site GUD was not related to NAO, however. This is easily explained by observing the link between NAO and local climate, since spatial correlations obtained by the Climate Explorer (Trouet and Van Oldenborgh 2013) among monthly NAO and monthly precipitation for the whole study period showed that winter precipitation in the western Iberian Peninsula was mainly controlled by NAO, mainly from October until March as stated by (Trigo et al. (2004)); this relationship was still relevant for April and May, but absent during summer. Therefore, the higher elevation and the different requirements for earlywood formation are probably responsible for a growth delay at GUD, so that earlywood vessel expansion occurs at a moment NAO is not affecting regional climate.

Conclusions

In this paper, we were able to establish climate-growth relationships for *Q. pyrenaica* across its distribution boundary towards the Atlantic region in northwestern Iberia. The use of earlywood anatomy and the amount of latewood proved to be a successful way to understand the effect of climate on this species, and both tree-ring compartments showed that climate played an important role on the radial growth of this species. In addition, the use of *Q. pyrenaica* appears to be promising for the study of climate change upon forest ecosystems, as it had strong a coherent signal, and it could be interpreted by the effect of NAO, a macroclimatic index. Therefore, we suggest that more knowledge on the ecology of tree species growing at boundary areas would be gained by studying this (or similar marcescent oak) species, probably complemented with understanding their wood formation dynamics.

Acknowledgements

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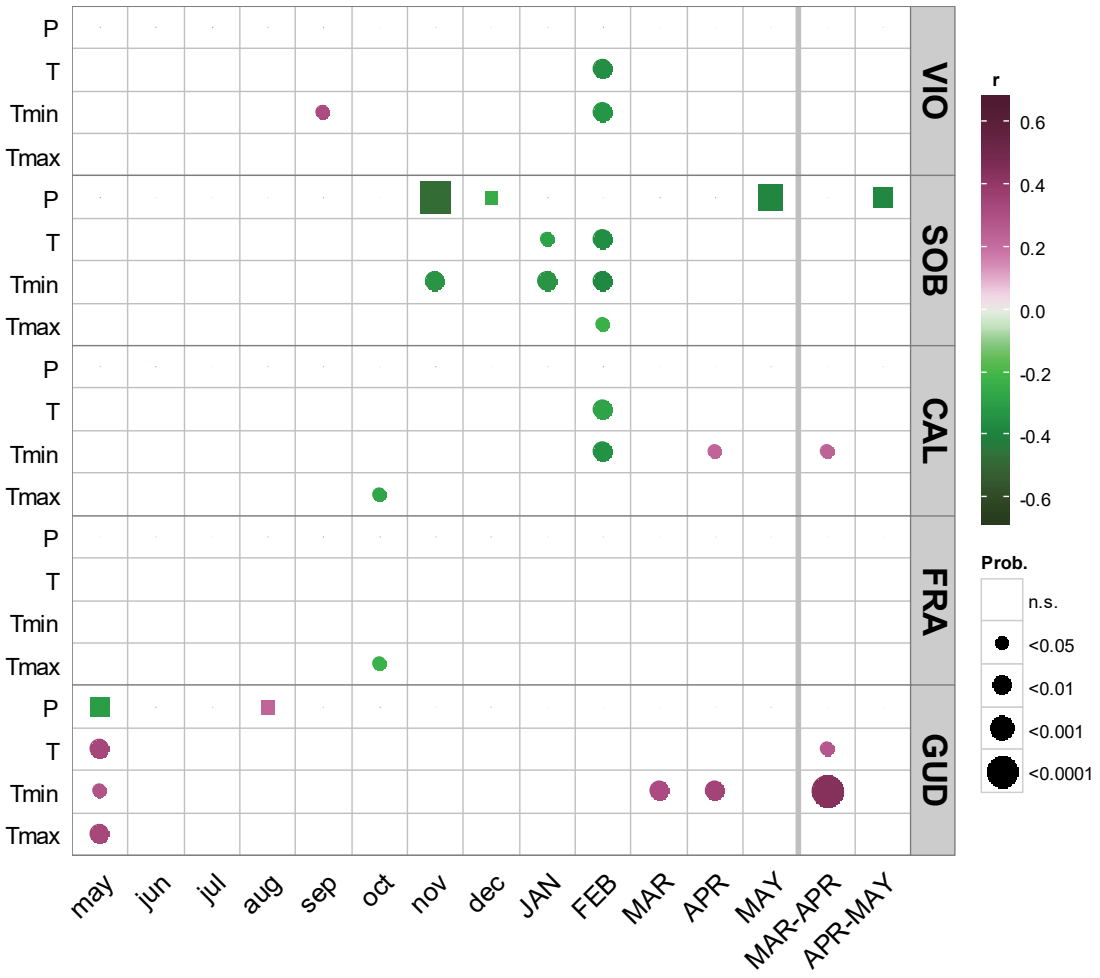
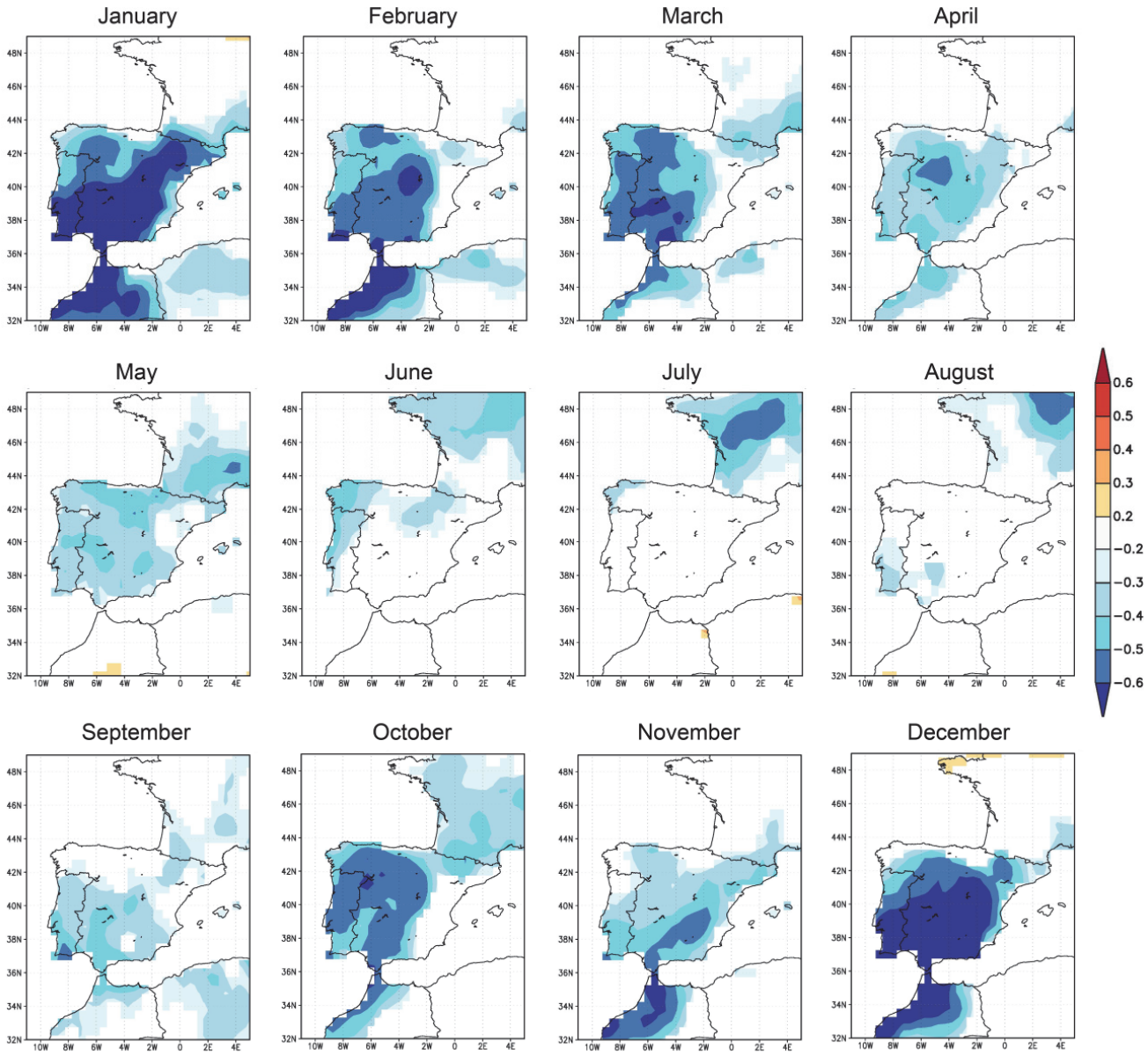
Supplementary material



S.1. Relationships to climate for mean vessel area (MVA), expressed as bootstrapped correlation coefficient, along the study periods 1953-2012, and 1967-2012 (only VIO). Lowercase and uppercase letters correspond to the months of the previous and current growth year, respectively.



S.2. Spatial correlations between mean monthly NAO-Gibraltar series and CRU TS3.22 monthly precipitation series for the period 1953-2012, p<10% for the Iberian Peninsula.

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ARTICLE V. AGE-DEPENDENCY AND TEMPORAL TRENDS IN A 481-YEAR LONG CHRONOLOGY OF EARLYWOOD VESSELS OF OAK

USC
UNIVERSIDADE
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Abstract
Earlywood vessels of ring-porous trees can be analyzed dendrochronologically, and used to obtain environmental information. However, most works deal with the analysis of climate-growth relationships at present, and do not evaluate their long-term variation through time.
We obtained a 481-year long chronology of oak (*Quercus robur* L.) in the northwestern Iberian Peninsula, using the size of earlywood vessels, compared it to a chronology of younger trees developed at the same site, and studied their trends through time. Earlywood vessel size was expressed as hydraulically-weighted diameter (HYD), and we separated vessels in the first row (*r1*) from the rest of the vessels (*nr1*).
There was very little variation in the response of trees belonging to both age classes. Old trees had more vessels belonging to the first row, and a lower common signal, but climate-growth relationships were practically the same. Spring temperature had a positive effect of vessel size, but only on the first row, probably mediating the onset of cambial activity. The chronology of old trees showed that age trend was nearly constant, except for the first decades, and common signal considerably varied through time, probably related to forest and climate dynamics. There was a strong negative correlation between the North Atlantic Oscillation Index (NAO) for winter and HYD, but the signal was not stable through time, mainly in the 20th century.
To our knowledge, this is the longest chronology of earlywood vessel size obtained so far, and it points out promising results, as this proxy does not appear to be age-dependent, and it was highly correlated to climate for long periods.

Keywords: Dendrochronology, tree ring, quantitative wood anatomy, time instability, age trend

Introduction

Tree rings are probably the most successful proxies for climate reconstructions due to their annual resolution (Esper et al. 2002; Jones et al. 2009). Most classical reconstructions were focused on the Alpine or boreal timberlines, and the tree-ring data typically used were ring width or maximum latewood density (Briffa et al. 2004; Briffa et al. 2002; Cook et al. 2004a) to reconstruct temperature, but many were also aimed at inferring past precipitation records (Cook et al. 1999; Cook et al. 2004b; Villalba et al. 1998), or even circulation models (Cook et al. 1998; Stahle et al. 1998). However, many works in the last two decades have shown that there are many other features in tree rings that can be used as proxies to obtain climatic or ecological information (McCarroll et al. 2003). Stable isotopes have been widely employed (Gagen et al. 2006; Loader et al. 2008; Saurer 2002), or more recently, blue intensity has been found to act as a surrogate for maximum density (Campbell et al. 2007). But the use of time series of anatomical features has also proven to be very useful to study tree adaptations to climate (Abrantes et al. 2013; Fonti et al. 2010; García-González and Eckstein 2003).

The study of anatomical characteristics across sequences of tree rings constitutes a link between dendrochronology and plant ecophysiology (Fonti et al. 2010; Gea-Izquierdo et al. 2012). As a result, they appear to be a valuable tool to study the adaptation of trees to global change by applying the so-called 'tree-ring anatomy' (Fonti et al. 2010). Annual anatomical variables also proved to be useful proxies in areas lacking a prevailing factor limiting growth (García-González and Fonti 2008), were 'classical' dendrochronology is often unsuccessful. Consequently, they have been recently applied to study the behavior of tree species in boundary distribution areas of temperate regions (Fonti and García-González 2004; González-González et al. 2014; González-González et al. 2015), contributing therefore to understand how these species could behave in a scenario of global warming.

Dendrochronological analysis of anatomical traits, i.e., annual quantitative values derived from one of several functional anatomical parameters, have been successfully applied to conifers (Bryukhanova and Fonti 2013; Olano

et al. 2013), diffuse-porous broadleaves (Abrantes et al. 2013; Oladi et al. 2014; Sass and Eckstein 1995), but appear to be especially promising for ring-porous species (Fonti and García-González 2004; Fonti and García-González 2008; García-González and Eckstein 2003; Gea-Izquierdo et al. 2012; González-González et al. 2014; Matisons et al. 2012).

Earlywood vessels of ring-porous species such as oak usually point out strong relationships to climate, despite having a low common signal in most cases (Fonti and García-González 2008; Fonti et al. 2007). But up to now, most of the research dealing with these traits has not been specifically focused at them in climate reconstruction. According to Bradley (1985), an ideal proxy must fulfill three characteristics, which include i) the presence of a significant relationship to the climatic variable that is to be estimated, ii) the existence of a coherent cause-effect relation, and iii) the persistence of a temporal stability. But most actual works on earlywood vessels have only been restricted to identify this relationship, and/or its ecophysiological interpretation. For example González-González et al. (2014) and González-González et al. (2015) compared different sympatric *Quercus* species which were sympatric, Fonti and García-González (2008) analyzed earlywood variation of oaks along a gradient, analyzed *Q. canariensis* along its distribution range, Matisons et al. (2012) provided information on the spatio-temporal variation of climate-growth relations of oak over a broad geographic area, or Gea-Izquierdo et al. (2012) analyzed *Q. canariensis* along its distribution range. Other authors highlighted the physiological significance of vessel variations. Thus, Fonti et al. (2007) attributed the negative response of vessel size to temperature before their formation to a modulation through sensitivity to auxin, or González-González et al. (2015) pointed out the importance of reserve consumption during dormancy; in addition, analyses of xylogenesis also added valuable information to understand the environmental control of vessel size (Pérez-de-Lis et al. 2015)

Although numerous recent papers support the two first characteristics of a good proxy, this is not the case for the third condition that must be fulfilled, as very few works dealt with long sequences of anatomical features, despite the existence of very long tree-ring sequences for ring-porous species such as oak (Friedrich et al. 2004). To our knowledge, there is just one investigation specifically aimed at long sequences (Fonti et al. 2009b), who analyzed a single chronology of ca. 350 years for *Quercus petraea*. This is probably due to the fact that the process of vessel measurement is still rather time consuming in comparison to other techniques, despite the existence of recent tools (Fonti et al. 2009a). In addition, the huge individual variation and therefore low common signal usually found in earlywood vessels (Fonti and García-González 2004; García-González and Fonti 2006), and the minimum amount of earlywood that should be measured (García-González and Fonti 2008) requires the analysis of a considerable number of samples.

Climate reconstructions are based on the 'Uniformitarian Principle' (Fritts 1976), which states that current patterns of tree growth must have been in operation in the past. These means that the validity of the relationships established depend on at least two circumstances, i) responses must have been stable through time, and ii) tree reactions to a factor must be independent of age. But there are evidences that this is not always the case, and this can hinder many climate reconstructions. Thus, several authors describe an instability of tree-ring relations to climate, mainly from the second half of the 20th century (Carrer and Urbinati 2006; Visser et al. 2010), which constitutes the so-called 'divergence problem' (D'Arrigo et al. 2008). On the other hand, may tree responses have been found to be age-dependent (Carrer and Urbinati 2004; Rozas et al. 2009a; Szeicz and Macdonald 1994).

In this paper, we develop a long chronology (481 years) of earlywood vessel size of *Q. robur*, and compare it to another series of younger trees obtained at the same site. The aims of our work are i) to determine if old and young trees are influenced by the same climatic factors, ii) to establish the variation of anatomical features through time, and iii) to evaluate if the relationship between long series and climate

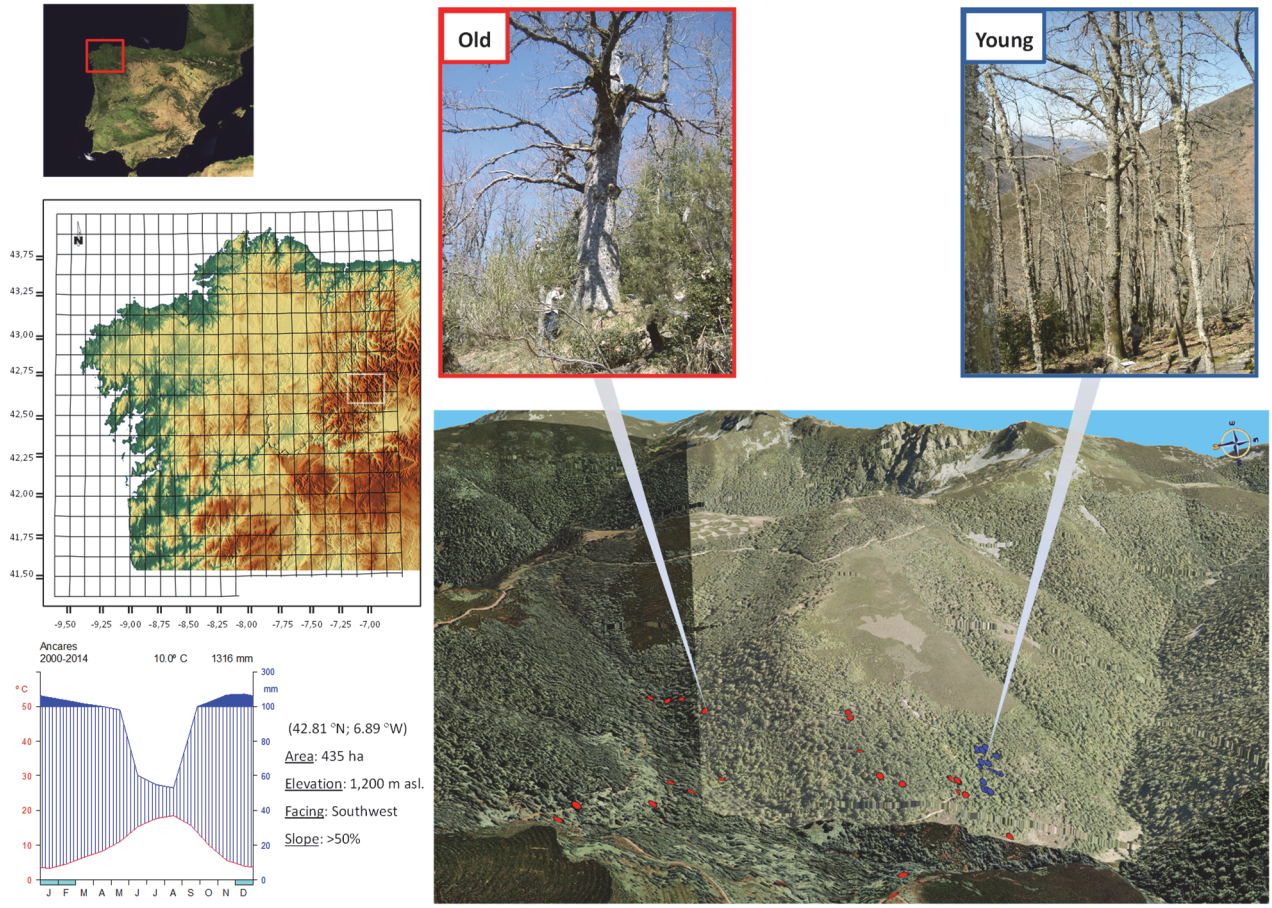

Fig. 1. Location of trees to obtain both chronologies over hillsides of Cabanavella, with older sampled trees portrayed as red points (*Old*), and younger as blue points (*Young*). Figure also shows a climate diagram from Ancares weather station, and the physiographic characterization of the study area.

could potentially be used for climate reconstruction.

Materials and Methods

Study area

We selected an oak stand at 1,200 m asl. at the western corner of the Cantabrian Mountains (Fig. 1), in Galicia, NW Spain (42.80-42.82 ºN, 6.88-6.92 ºW). This forest, known as Cabanavella, lies very close to the altitudinal distribution boundary of oaks in the region, and is dominated by *Quercus robur* and *Q. petraea*, which have been described to potentially hybridize in this region (Vila-Lameiro and Díaz-Maroto Hidalgo 2002). The oak woodland covers a large extension (435 ha) within the mountain range known as Ancares. It constitutes one the oldest forests in the region, and it extends over hillsides on deep soils, where ancient oaks are scattered with younger trees under low human pressure at present, but having suffered from an intense logging in the past until the second half of twentieth century. Natural oak forest in this area correspond to the Orocantabrian association *Linario triornithophorae-Quercetum petraeae* (Rivas-Martínez 1987), where oaks (*Q. petraea* an *Q. robur*) are dominant, but can be accompanied by other broadleaves such as *Betula alba* L., *Acer pseudoplatanus* L., *Sorbus aucuparia* L., *Corylus avellana* L., or *Ilex aquifolium* L.

Climate regime is Atlantic, but having the harsh characteristic of mountain environments. Annual precipitation is high (1,600-1,800 mm), mainly concentrated during autumn and winter (often as snow); convective storms can often occur in spring on summer, and therefore summer drought is absent in most years. With a mean annual temperature ca. 9 ºC, summers are

mild (15 ºC), and winters are cold (4 ºC), with a long frost period, and frequent fogs and thermal inversions in the valleys. As a result, vegetation period can extend for no more than six months a year.

Within this woodland, we performed sampling on slopes facing South-Southwest, where collected two different typologies of trees (Fig. 1). On the one hand, we sampled the largest and apparently oldest trees (hereafter '*Old*'), widely dispersed and occasionally accompanied by *Acer pseudoplatanus, Ilex aquifolium,* and *Betula alba,* with the aim on obtaining a chronology that could expand a few centuries. On the other hand, mature but considerably younger trees (<100 years old) were selected on a single slope apparently even-aged from natural regeneration (hereafter '*Young*'), and with a poor understory mostly dominated by *Erica australis*.

Sampling, wood processing, and anatomical measurements

We extracted at least two 5-mm increment cores per tree from selected dominant individuals belonging to both age classes, namely 27 for *Old*, and 18 for *Young*. Samples were air-dried, glued onto wooden supports, surfaced using a WSL sliding microtome (Gärtner and Nievergelt 2010), and finally polished with progressively finer sandpaper (grain sizes from P220 to P1200, FEPA (Federation of European Producers of Abrasives) Abrasives). We removed tyloses and wood dust were using high-pressure water blast (Fonti et al. 2002), stained core surface with black printer ink, and applied chalk to fill earlywood vessels (González-González et al. 2014), gaining therefore an optimal contrast for image analysis.

We measured earlywood (EW) and latewood width (LW) using a tree-ring measuring linear stage (Velmex TA UniSlide, Velmex Inc., Bloomfield NY, USA) coupled to a binocular microscope (Olympus SZ60) at 20-40x magnification. We considered earlywood as the portion of the ring that was exclusively composed of large vessels forming a continuous band (García-González and Eckstein 2003), without the fiber tissue that is characteristic of latewood. We visually crossdated the series of total ring width (RW), obtained as the year-to-year sum of EW and LW, by comparing individual curves; we assessed statistical verification by means of COFECHA (Grissino-Mayer 2001).

Vessel measurements were performed on a subset of 12 trees for *Old*, and 13 trees for *Young*, selected out of those with scarce problems for vessel measurements, and a good correlation to the local chronology. The cores were digitized using a digital photo camera Canon EOS 600D, incorporated to a binocular microscope Olympus SZ60, and progressively moving the core by a mechanically driven platform. We obtained sequences of high resolution images (5184 x 3456, 17.9 Mpx) with a common overlap region, which were subsequently merged into one single picture for core, using PTGui ver. 9.1.8 Pro (New House Internet Services B.V., Rotterdam, The Netherlands), and saved into a TIFF file.

Earlywood vessels were automatically detected and measured in ImageJ (Abramoff et al. 2004; Schneider et al. 2012), using the VesselJ plugin (García-González, not published). Image segmentation was performed on an 8-bit threshold, as gray levels were enough to clearly discriminate bright earlywood vessel lumina from a dark the background. But the presence of undesired object detected as vessels was common in all images, and therefore we had to eliminate these objects before storing the values. This was done by means of automatic procedures (removing vessels smaller than 10.000 μm^2 and twice longer than wider), and by manual corrections of the image. An erode-dilate operation (2x2, one pass) and the adjustment of the convex hull improved vessel outlines in a final step.

Each core was analyzed as a single image, and the allocation of all vessels to the corresponding dated tree ring was accomplished by the application of the software Autovasos (García González, not published), specifically designed to ring-porous species. We separated vessels belonging to the first row from those located later in the earlywood during the process of vessel assignation; vessels close to the tree-ring boundary, and those whose beginning did not

exceed the imaginary line connecting the centers of the ring boundary vessels, were considered as vessels in the first row. The correct calendar year was rechecked through the comparison between the dated tree-ring series and the series of ring width automatically calculated by Autovasos.

Analysis of vessels and chronologies

We tested if vessel size distribution was related to the tree age. For this, we analyzed individual tree distributions for the common period 1938-2003, looking for differences between young and old trees. We also studied age trend within vessel size using the *Old* subset (1523-2003). For this, we aligned trees along with cambial age and not calendar year, but as the pith was not present in cases, we needed to estimate the number of missing rings. Age estimation in partial cores containing the arcs of the inner rings followed the method proposed by Rozas (2003), whereby pith location and missing rings are estimated by the combined use of a graphical method and a specific equation. If the arcs were not present, the length of missing radii was calculated as the difference between the stem geometric radius and the total core length, computing the number of missing rings by extrapolating the mean radial growth rate from the innermost 20 rings in the cores. Once aligned, distributions of vessels were studied by pooling data for all trees in the same age class, using 5-year periods. All analyses were performed for the whole earlywood, as well as separating vessels by row.

As regards chronologies, we initially studied 15 variables, including widths, namely earlywood (EW), latewood (LW), and the whole ring (RW), and 12 vessel variables. All vessels from increment cores of the same tree were pooled, and their statistical distribution used to calculate these numerous earlywood variables out of the initial dataset of vessel measurements. For all vessels, we considered the total number of vessels (NV); total vessel area (TVA); mean vessel area (MVA); mean area of vessels larger than 90th percentile of each ring (M90); hydraulically weighted diameter (HYD = $\sum_1^n d_t^5 \,/\, \sum_1^n d_t^4$, for *n* vessels of diameter *d* in year *t*) proportional to xylem hydraulic conductivity (Fichot et al. 2009; Sperry et al. 1994), and the specific conductivity (KS) of the earlywood (kg m^{-1} s^{-1} MPa^{-1}) in year *t* according to the H-P law (Tyree and Zimmermann 2002), so that KS = $(\pi\varrho/\,128\eta A)\sum_1^n d_t^4$, where ϱ is the density of water at 20 ºC (998.2 kg m^{-3}), η is the viscosity of water at 20 ºC (1.002 x 10 $^{-9}$ MPa), A is the area of the earlywood analyzed in yeat *t*, and d_t is the diameter of each of the *n* vessels measured in year *t*. NV and TVA were standardized to a frame of 10 mm of tangential width, whereas the conductive area to obtain KS was automatically calculated as the area of the convex polygon including all vessels. In addition, we also considered size variables (MVA, M90, and HYD) for vessels belonging to first row (***r1***) or not (***nr1***) separately, because the selection of largest conductive elements at the beginning of the season usually improves the climatic signal (García-González and Fonti 2006).

Individual series were standardized by a 32-year cubic smoothing spline (Cook et al. 1992) with 50% cutoff. This spline is optimal for young trees, but would have the risk of removing low-frequency cycles in old oaks. However, the study of such cycles is out the scope of our paper, so we preferred to keep the same detrending method for all series for the sake of comparison. Furthermore, as trends in the anatomical series were in general smooth, the curve adjusted during standardization should hardly impact the results. We obtained the time series of growth indices by dividing each observed value by that estimated by the spline function (Fritts 1976), and we averaged the indices into a chronology for each variable and site, using a biweight robust mean (Mosteller and Tukey 1977) to minimize the effects outliers. Autorregression modelling was not necessary, as most autocorrelation was due to trend, and therefore successfully removed by spline filtering.

After detrending, we evaluated *Old* and *Young* chronologies for quality in their common signal, by choosing a common period for both (1938-2003, 66 years). We used the mean correlation between trees (*Rbt*), which is the mean of all possible cross-correlations between individual

tree series; and the express population signal (*EPS*), a parameter indicating to what extent the chronology sample represents the whole population. Mean sensitivity (*MS*) and the first-order autocorrelation coefficient (*AR1*), where also used to respectively evaluate the year-to-year variation, and the effect of the previous year on actual growth. We also assessed the variation of these statistics through time for the *Old* chronology. For this, we calculated the parameters defining common signal for 50-year periods shifted in one year, and from 1626-2003 (378 years), which was the period covered by at least six trees.

We did not need to compare these variable chronologies to select the most appropriate for studying the effect of climate, as a previous investigation involving this site suggested that LW, HYD-r1, and HYD-nr1 were the variables with the highest potential. However, we studied the variations in the relationships between these variables along the period 1732-2003 (at least eight trees for *Old*) using principal component analysis (PCA), which proved to be very useful at identifying groups in time series of anatomical variables (Bryukhanova and Fonti 2013; González-González et al. 2014; Kniesel et al. 2015). PCA was applied on the correlation matrix of all variable chronologies for time lags of 50 years shifted in one year, and the evolution of the relations among variables was identified by the loadings on the two first principal components following varimax rotation.

We also used varimax-rotated PCA to analyze the ordination of individual trees for the selected variables (HYD-r1, HYD-nr1, and LW) so as to visualize the differences between age groups. This analysis was performed on the common period 1938-2003.

Climate-growth relationships

We performed two different types of analysis to compute climate-growth relationships. On the one hand, we compared variable chronologies of each subset of trees (*Young* and *Old*) to climatic data sets for the common period 1938-2003, in order to test possible differences in the response as related to tree age. On the other hand, we used the chronologies of *Old* trees to study the climatic response over a longer period, and also the likely variation of climate-growth relationships through time.

As no continuous and reliable weather records were available for the eastern Galician mountains, we used the gridded CRU TS 3.22 dataset (University of East Anglia Climatic Research Unit et al. 2014) of monthly precipitation and temperature (mean, minimum, and maximum) available at the KNMI Climate Explorer (http://climexp.knmi.nl/) for the period 1901-2013, with 0.5º × 0.5º of geographical resolution. This data set served to investigate the compared relationships of *Old* and *Young*. For the specific analysis of old trees we employed monthly indices of the North Atlantic Oscillation (NAO), a measure of the difference in the pressure fields between the Icelandic Low and the Azores High that considerably influences weather in western Iberia, especially during winter (Trigo et al. 2004). NAO data for the Gibraltar observatory were directly available from the CRU database (http://www.cru.uea.ac.uk/cru/data/nao/), spanning for the period 1825-2015. This was the only reliable climatic record that could be suitable for the study site with availability for such a long period, and it allowed us to study the long-term effects of macroclimatic records.

We calculated bootstrapped Pearson's correlations between index chronologies (LW, HYD-r1, HYD-nr1), and meteorological records along the growing season. Significance of the correlation coefficients was computed by the percentile confidence intervals of 10,000 bootstrap iterations, following the correction proposed by Mason and Mimmack (1992) for non-normally distributed estimators of the coefficient. Growing season was established from previous to current May for earlywood variables, and to current September for latewood; apart from monthly values, we seasonalized data for spring (April-May) and winter (December-January and December-February) for HYD, and late winter-early spring (February-April) for LW.

Temporal analysis for the old trees was done along the period 1825-2003, which was covered

by at least nine trees. For this, we calculated moving correlation functions as defined by Biondi and Waikul (2004), using 45-year periods, shifted in one single year. We used monthly NAO indices from previous May to current October, as well as seasonal values for early winter (December-January). This analysis was aimed at studying the stability of the observed relationships along the last two centuries. A correlation value for the whole period was also calculated.

Results

Vessel-size distribution

Due to the different age classes, the amount of all measured vessels was more than the double at *Old* (*n*=96,434) than at *Young* (*n*=41,949), and consequently these numbers are not comparable (Fig. 2). However, there were no remarkable differences between both distributions, neither for *r1* nor for *nr1*. Whereas distributions in *r1* were only slightly skewed, and tended to normality, those for *nr1* had a clearer prevalence of smallest vessels, though not extremely asymmetric.

Mean vessels area was slightly higher for *Young* than for *Old* (58,947 μm^2 vs. 56,979 μm^2 for *r1*, and 36,071 μm^2 vs. 34,483 μm^2 respectively). Only the proportion of vessels in *r1* was considerably higher for *Old* (54.6% vs 36.2%), probably due to sequences of reduced growth, where many rings just presented a single vessel row.

Old allowed the study of the variation of vessel size along with cambial age, which has been calculated for a time period of 500 years (Fig. 3). Vessels were much smaller in young trees, but this age trend was only present for the first 20-30 years, and is probably related to the presence of juvenile wood. Afterwards, vessel size stabilized, and there was no clear trend for most of life span of the tree. All vessels and those in *r1* clearly had the same trend, but vessels in *nr1* got larger until a cambial age of ca. 100 years, and the trend kept rather steady afterwards. The ascending vessel size for ages

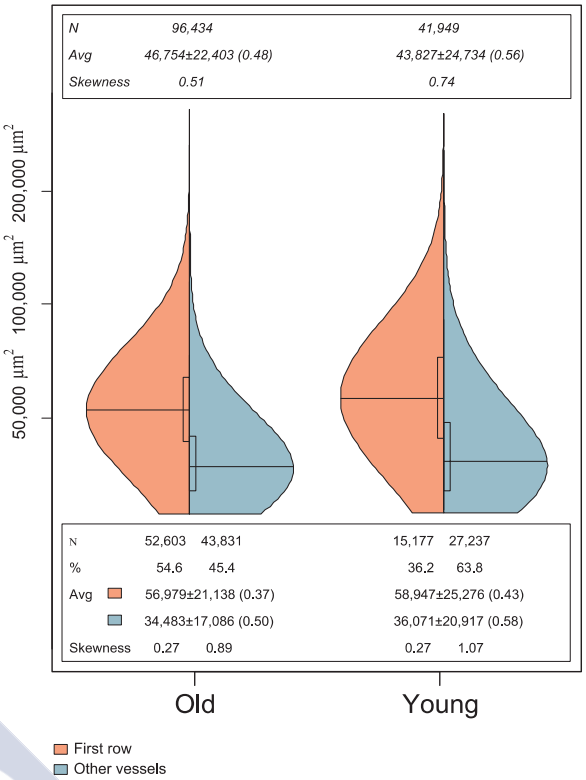

Fig. 2. Violin plots of each subset of trees, and corresponding descriptive statistics for all measured vessels (italics), and for separated rows. *N*: number of vessels; *Avg*: mean vessel area (μm^2) ± standard deviation (coefficient of variation).

more than 450 years, especially visible for *r1*, were probably due to the small number of trees that reached that period, and should be of no importance.

Study of the relationships among variables through time

Preliminarly, the ordination of all 15 study variables on the space defined by the two first principal components did not show remarkable differences between *Old* and *Young* trees along the common period 1938-2003 (Fig. 4).

Multiple PCAs calculated for 45-year periods along 1732-2003 clearly allowed us to study how these relationships among variables varied through time (https://dendrosite.wordpress.com/), indicating that this pattern was very robust, and did not change through time dramatically. As previously observed, there were three clear groups, namely i) LW and RW clearly separated along the first component; ii) variables related to *nr1*-vessels

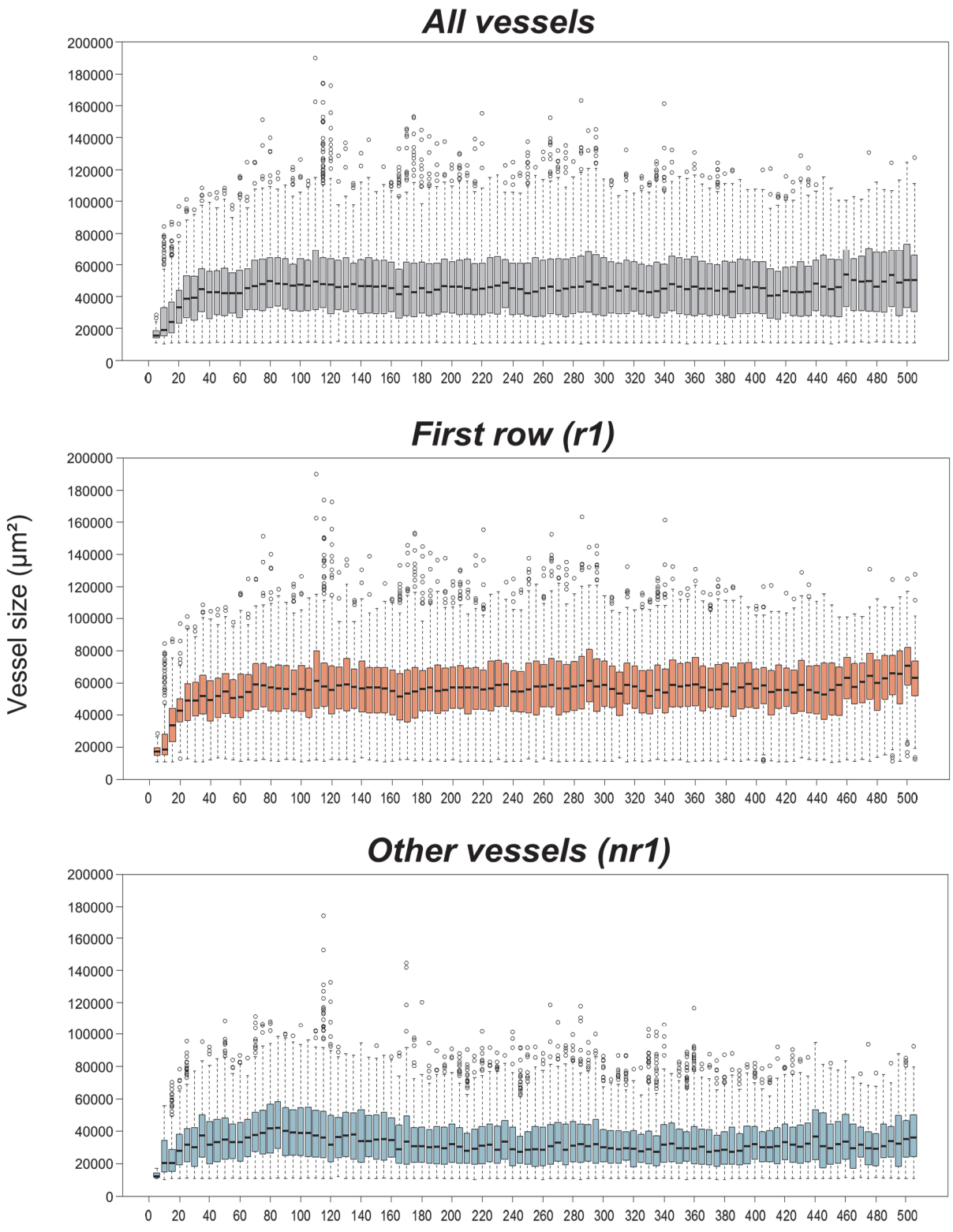

Fig. 3. Boxplots of the *Old* chronology (1523-2003) representing all vessels, first-row vessels (*r1*) and the other earlywood vessels of the ring (*nr1*), aligned along their cambial age. Values were grouped in 5-year periods.

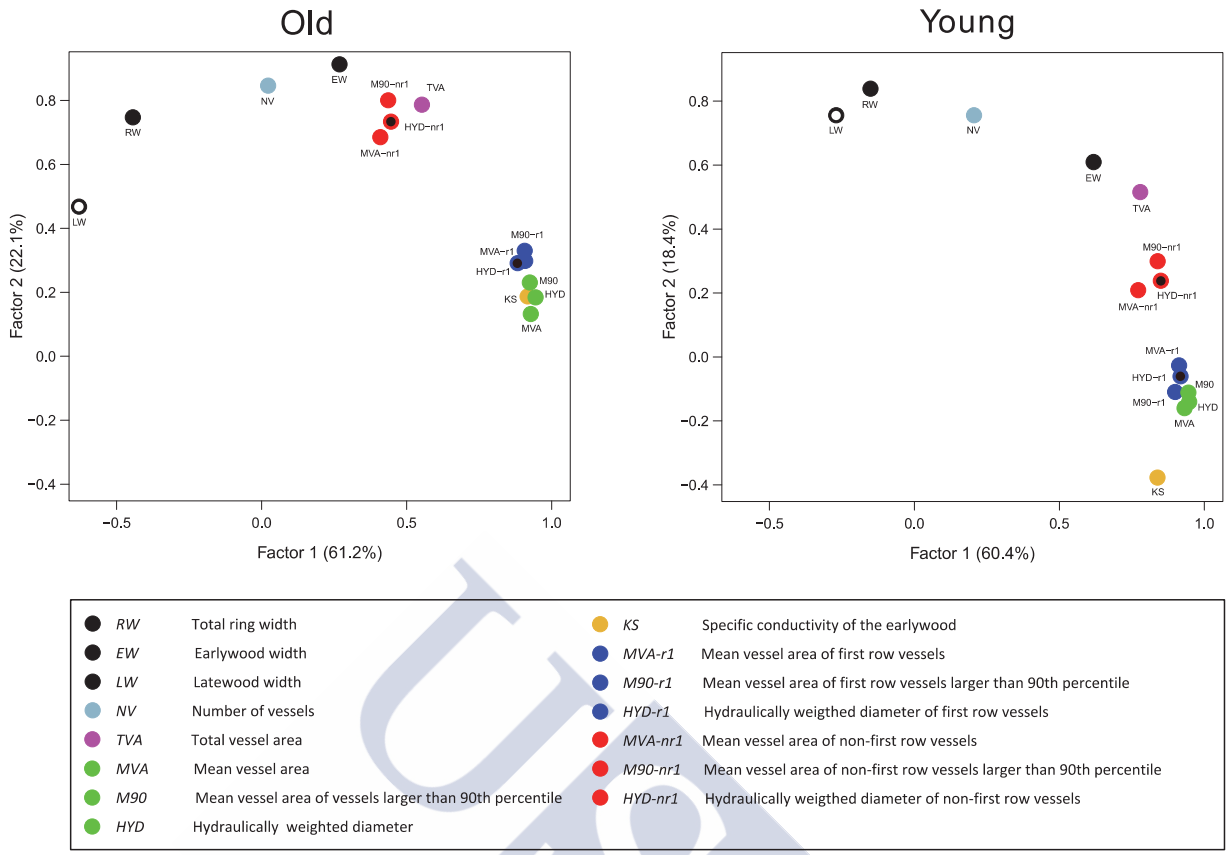

Fig. 4. PCA of preliminary study of variables for both chronologies along the common period 1938-2003, highlighting the selected ones.

and TVA; and iii) those including all vessels (MVA, HYD, M90, KS) together with *r1*-variables. NV and EW were somehow 'intermediate' variables along the first eigenvector, situated between the width variables and the *nr1*-group, and their location showed more variation through time; NV was always more related to LW and RW, whereas EW was linked to the *nr1*-variables. Only the relations between variables at *r1* and *nr1* did change between approximately 1840 and 1900. This period included very narrow rings, often having only one row of earlywood vessels; after this period, the relationships among variables recovered the original trend.

Comparison between chronologies of age classes

Chronologies of both groups of trees were very similar to each other for the common period of overlap, and there was a very consistent match between them (Fig. 5). Correlations were however higher for by LW (r=0.83) than for the earlywood (r=0.69 for HYD-*r1*, and r=0.71 for HYD-*nr1*), so that HYD probably registers more differences between age classes than LW. In addition, correlation between HYD-*r1* and HYD-*nr1* was similar (r=0.57 for *Old*, and r=0.64 for *Young*), indicating that the relationship between both earlywood compartments was not age-dependent (Fig. 6a).In fact, this relationship slightly changed through time, but this related to ring width rather than an age trend. The highest correlation was reached between 1840 and 1900, a period of very reduced growth, when a single vessel row was not exceptional for a considerable number of rings (Fig. 6b).

The ordination of all trees covering the common period on the space defined by the two first eigenvectors of a PCA showed that they behaved as a single population, regardless of the age and variable considered (Fig. 7); only a slight tendency could be observed with LW, but still very feeble.

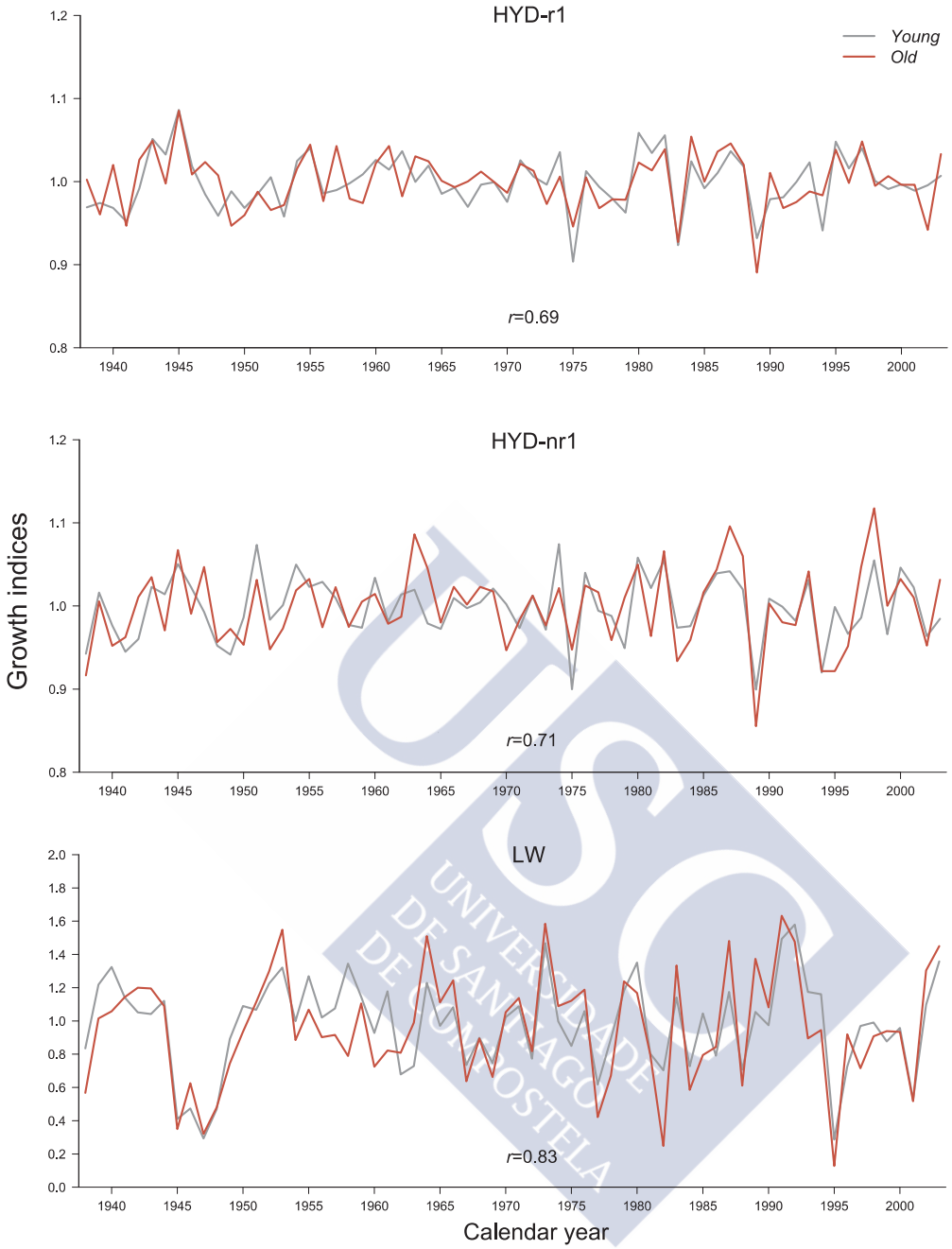

Fig. 5. Comparison of the chronologies *Young* and *Old* trees, for the hydraulic diameter of the vessels in the first row (HYD-r1), non-first row vessels (HYD-nr1), and latewood width (LW), along the study period 1938-2003.

Earlywood chronologies showed much lower statistical quality than LW for the common period (Table 1). LW fitted within the thresholds recommended by Wigley et al. (1984), with *Rbt* higher than 0.35 (0.44 for *Old*; 0.51 for *Young*), and *EPS* over 0.85 (0.90 and 0.91 respectively). Yet, the low values registered for HYD were considerably higher than usual for earlywood variables, with the best quality for the HYD-r1 chronology of *Young* (*Rbt*=0.34, *EPS*=0.84), which was within values that are usually found for ring-width chronologies. Notwithstanding the lower common signal, all statistics evidenced the existence of a common pattern within the earlywood vessels that deserves to be studied dendrochronologically. Year-to-year variability was very low for HYD-r1 and HYD-nr1 (*MS*=0.03-0.05), and considerably high for latewood (*MS*=0.30-0.39). As regards persistence, chronologies were not

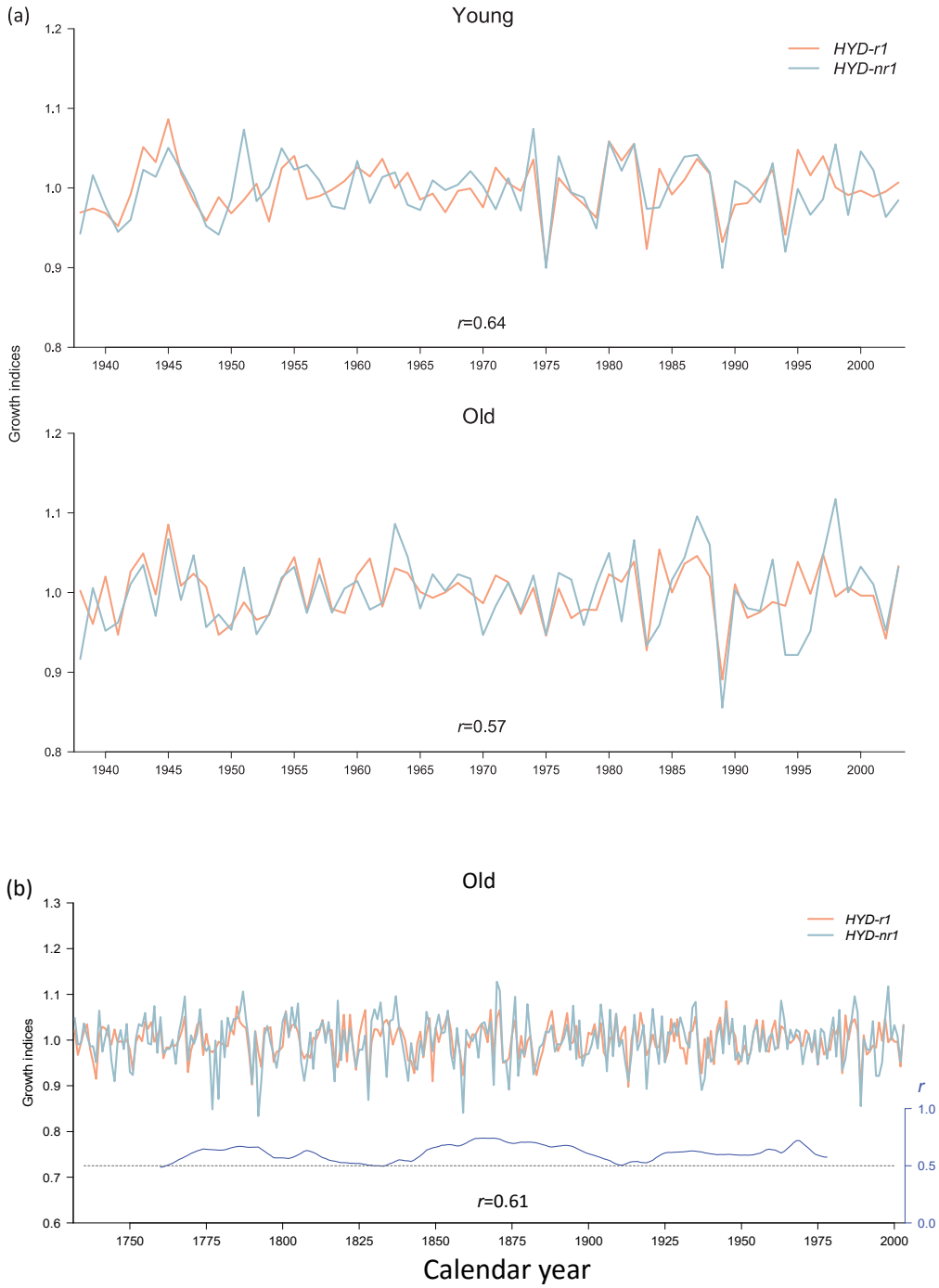

Fig. 6. Comparison of the chronologies of hydraulic diameter of the vessels in the first row (HYD-r1), non-first row vessels (HYD-nr1)for *Old* and *Young* subsets along the period 1938-2003 (a); and only for *Old* along the period 1732-2003 (b); the blue line represents the correlation between HYD-r1 and HYD-nr1 (50-year periods).

autocorrelated, except weakly for LW at *Young* (*AR1*=0.29).

Common signal showed some variation through time (Fig. 8), probably due to i) the changing environmental conditions for the last centuries, ii) the fact that new trees covered the chronology towards recent periods, and iii) also because age trend was not the same for all variables. *Rbt* for LW increased approx. between 1725 and 1840, and then lowered from ca. 1880 with a slight recovery in the most recent decades; but the trend for *Rbt* of earlywood (mainly HYD-r1) was nearly reversed until the mid 19th century. *EPS* remained steady or raised through time, because the number of

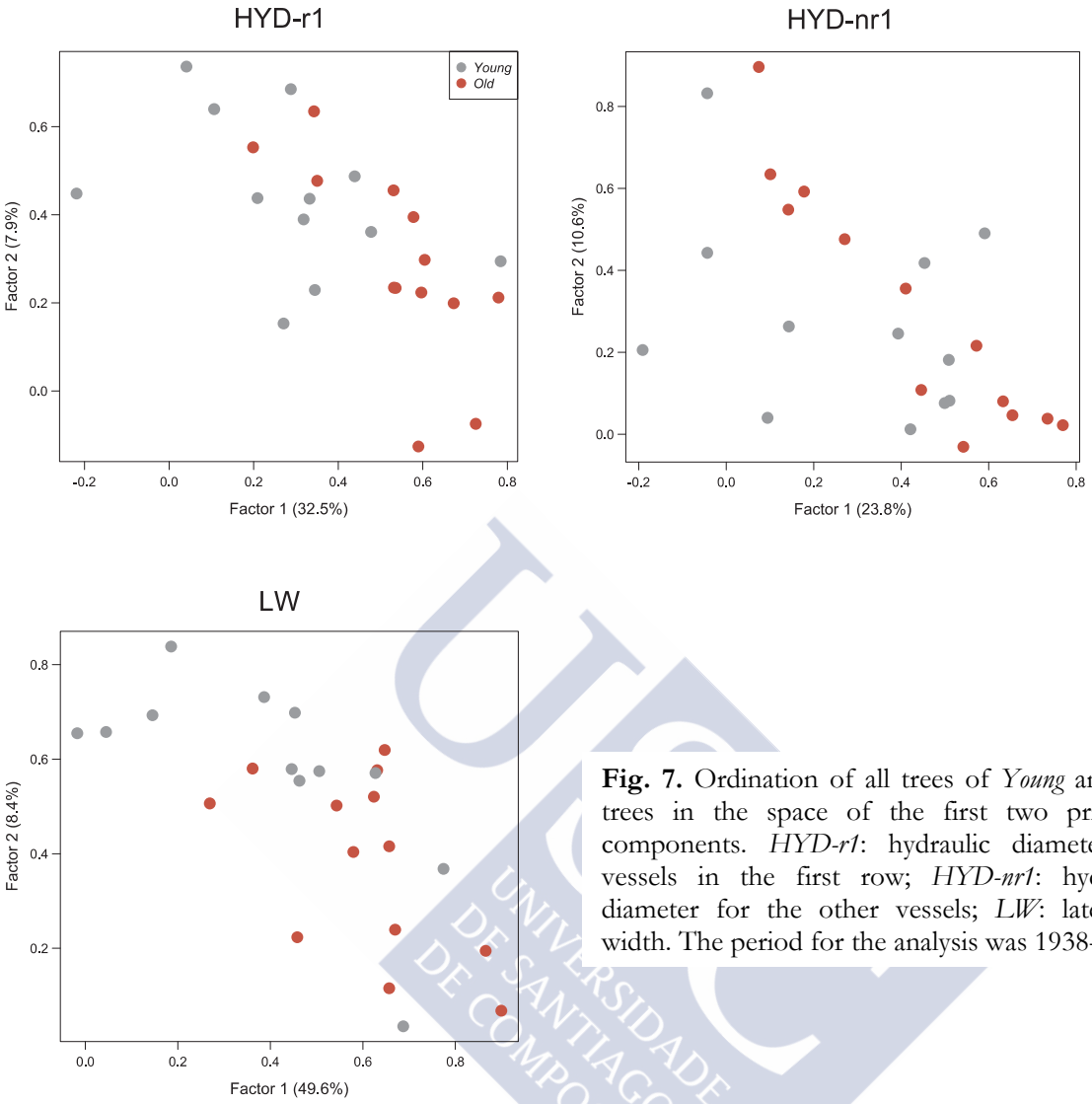

Fig. 7. Ordination of all trees of *Young* and *Old* trees in the space of the first two principal components. *HYD-r1*: hydraulic diameter for vessels in the first row; *HYD-nr1*: hydraulic diameter for the other vessels; *LW*: latewood width. The period for the analysis was 1938-2003.

trees was larger towards the end of chronology, so it somehow buffered the effect of the changing *Rbt*. *MS* of the earlywood vessels was nearly steady, whereas that of LW exhibited notable variations along the whole period, probably related to mean ring width. Finally, *AR1* had a similar behavior for LW and HYD-nr1, which was opposite to HYD-r1; it increased from the beginning of the 17th century, and had a important drop in the second half of the 18th century, when that of HYD-r1 attained its maximum. This coincides with the period of very narrow rings, with few vessel rows and nearly no latewood; recovery was strong afterwards. Nevertheless, values of *AR1* were not high for most of the chronology length, especially for HYD.

Climate growth-relationships

The relations to climate were almost indentical for young and old trees, but in general we observed few significant correlations. A positive effect of spring temperature was recorded only by HYD-r1 around April (Fig. 9), mainly driven by maximum temperature for *Old* (r=0.41 P<0.001), and by mean temperature for *Young* (r=0.45, P<0.001), with lower correlations to minimum temperature. Additionally, young trees also showed a certain effect of the precipitation of the previous summer, namely June (r=–0.37, P<0.01), and old trees were weakly related to spring precipitation (r=–0.26, P<0.05). We found nearly no relations for HYD-nr1.

Table 1. Chronology quality for *Old* and *Young* trees, for the common interval 1938-2003.
Rbt: mean correlation between trees, *EPS*: expressed population signal, *MS*: mean sensitivity, *AR1*: first-order autocorrelation coefficient.

Chrono	HYD-r1		HYD-nr1		LW	
Plot	Old	Young	Old	Young	Old	Young
Rbt	0.24	0.34	0.15	0.21	0.44	0.51
SNR	3.83	5.12	2.19	2.40	9.36	10.54
EPS	0.79	0.84	0.69	0.71	0.90	0.91
MS	0.04	0.03	0.05	0.04	0.39	0.30
AR1	-0.12	0.02	-0.04	-0.17	0.16	0.29

Total length of chronology: 1520-2003 (*Old*), 1905-2004 (*Young*)
Trees covering common interval: 12 (*Old*), 10 (*Young*)

Responses for LW were less significant than for HYD-r1 (not showed), and mainly consisted of an inverse relationship to temperature from February to April (r=–0.29, P<0.01, with mean temperature), and positive to late summer temperature (r=0.30, P<0.01, with August, when using minimum temperature) both for *Old*; these results, though weak, were only observed for the subset of old trees.

Correlations between the North Atlantic Oscillation (NAO) and the oldest trees along the period 1825-2003 (Fig. 10) were significant, and spread to all earlywood vessels. This relationship was clearly evident for the winter months, maximized by the cumulative period December-January, (r=–0.34, P<0.0001 for *r1*; r=–0.38, P<0.0001 for *nr1*; n=178 years), and only slightly lower for December-February (r=–0.33, P<0.0001 for *r1*; r=–0.34, P<0.0001 for *nr1*; n=178 years). LW did not show any significant correlation to NAO for any studied period.

But the study of the trends of these responses to NAO since 1825 to 2003, in 45-year periods, revealed that the signal was not completely stable, and there were two opposite patterns (Fig. 10). HYD-r1 presented an ascending trend until the period 1890-1935, when the maximum correlation was reached (r=–0.50, P<0.0001), and from this point the correlations decreased rapidly, becoming non-significant for most of the 20th century, though recovering during the last decades. On the other hand, the trend observed for HYD-nr1 was descending, with a progressive signal loss that reached its minimum at the same moment as HYD-r1, and it also increased recently to recover the initial values.

Discussion

We assessed the comparison of trees belonging to both age classes using two different approaches. On the one hand, we compared the responses of young and old trees at present, using the common period 1938-2003; and, on the other hand, we just analyzed old trees in order to identify the variation in their trends through time. But both approaches need to be interpreted together to understand the role of age driving the responses of earlywood anatomical traits.

A correct comparison of *Young* and *Old* trees growing at the same site would require that all potential variables other than age are minimized. This is not an easy task in a natural forest, and presented some difficulties in our case due to the heterogeneity within the woodland. In fact, our sample of *Young* was much more homogeneous, as most trees came from a small even-aged forest patch, whereas *Old* were considerably scattered all throughout the forest. But despite this, we hardly find differences between both groups, and the ones found could also be more related to microsite differences, and not only age. One of the main evidences of the similarity between age classes was found in the ordination of individual trees, as none of the analyzed variables (LW or HYD for rows) showed a clear separation between both age classes, but only a weak trend; besides, the result was the same even if further earlywood variables were considered (data not shown), confirming the parallelism between *Young* and *Old*.

Characteristics of vessels and anatomical variables

Most differences between age groups corresponded to the distributions of vessel size, in particular with regard to the distribution into vessel rows, and did not influence further

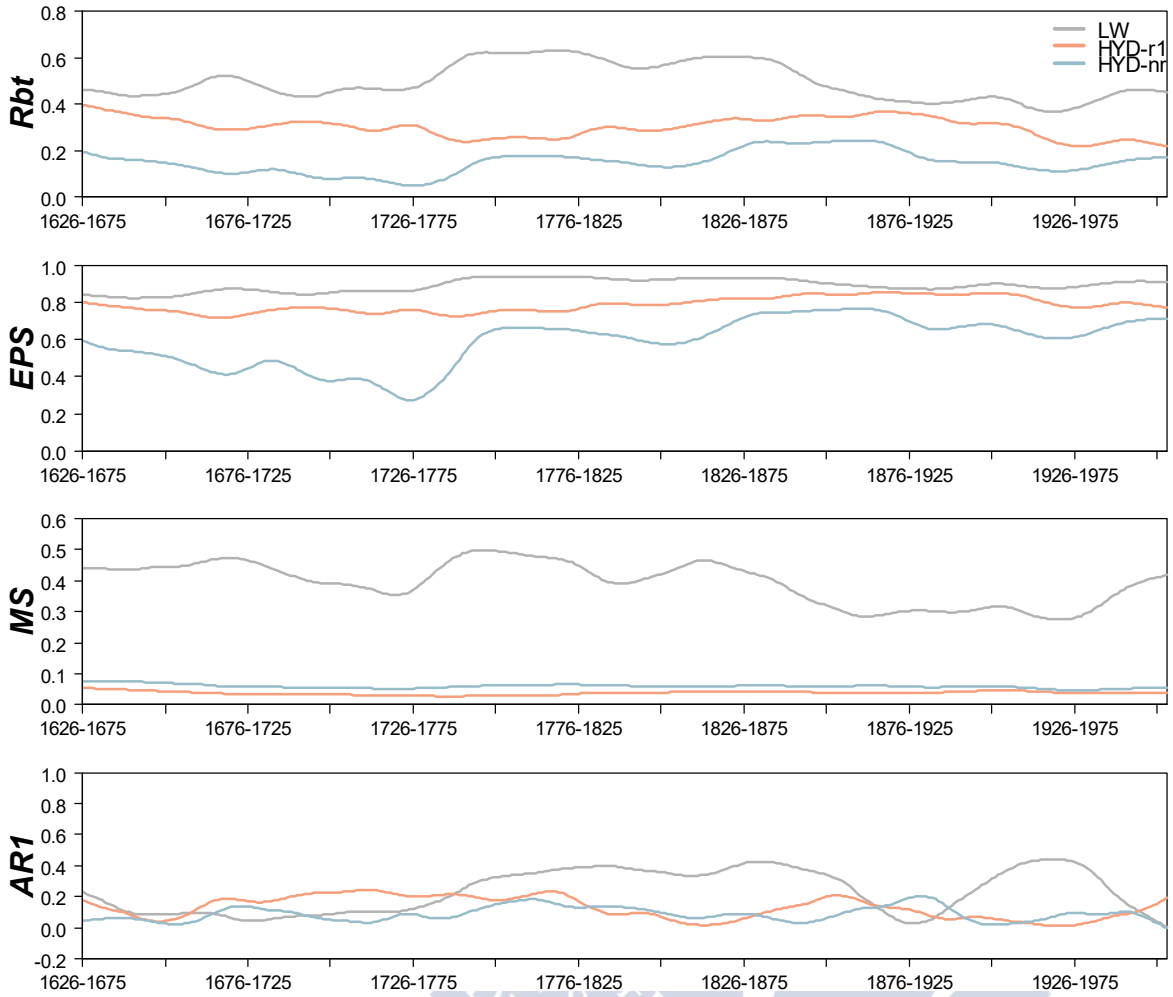

Fig. 8. Variation through time of the statistics of the common signal, calculated on a 50-year moving window, for the Old chronologies along the study period 1626-2003. Trend lines were smoothed using spline interpolation. *HYD-r1*: hydraulic diameter for vessels in the first row; *HYD-nr1*: hydraulic diameter for the other vessels; *LW*: latewood width; *Rbt*: mean correlation between trees, *EPS*: expressed population signal, *MS*: mean sensitivity, *AR1*: first-order autocorrelation coefficient.

analyses once the chronologies were computed. Vessels were only slightly larger for *Young*, in both the first row and the result, but these differences were negligible. Surprisingly, *Old* appeared to have larger vessels when considering the whole pool instead of the rows, confirming the convenience of using vessel rows to gain more information (García-González and Fonti 2006; González-González et al. 2014). But this apparent discrepancy was only caused by the proportion of vessels within each earlywood compartment, which was considerably higher for the first row (the largest vessels) for *Old*, with many rings having only one or two rows. In contrast, *Young* exhibited more radial increment, with several rows of smaller vessels. Furthermore, size distributions by row confirm that these differences were only due to the proportions of vessels per row, because they were similar for both age groups; they tended to be normal for the first row, and clearly dominated by small vessels for the rest. But to our understanding, these results only reflect the age trend that is present in these anatomical series.

It is well-known that the earlywood vessels of ring-porous trees bear a characteristic age trend, which affects vessels in their size, number, or even spatial distribution (Gasson 1987). Vessels at the beginning are small and forming several rows (Helinska-Raczkowska 1994), but they get

larger along with age, and restricted to one to few rows. According to Petit et al. (2008), lumen diameter of the conduits increases with cambial age at a rate that depends on stem elongation. However, this ascending trend is not constant, but it is nearly stabilized after a few decades, which is coherent with the fact that trees approach their maximal height soon after maturity (Husch et al. 2003). This was very clear in our data for *Old*; when we aligned vessel size along with cambial age, the trend was practically absent, even as trees were rather young, so that the period with a clear ascending trend only spanned for 30-40 years, which seemed to correspond to juvenile wood (Helinska-Raczkowska 1994; Helinska-Raczkowska and Fabisiak 1999).

Furthermore, the trend was similar regardless of the row considered, although vessels not in the first row displayed slightly more variation, especially immediately after the juvenile period. But the trend for earlywood vessels was not essentially different from that found for the conduit size of most species, regardless of the anatomical type (Carrer et al. 2015). However, our comparison between both age groups did hardly involve juvenile wood, as many of the rings were already located within what we can consider 'mature' wood; this is especially relevant for the analysis of time series, because it was restricted to the common period 1938-2003, assuring a considerable number of trees, but still avoiding the presence of juvenile wood for *Young*.

The existence of this trend has been previously mentioned in dendrochronological analyses of vessels. (Gea-Izquierdo et al. 2012) stated that tree rings of *Q. canariensis* appeared to be semi-ring porous rather than ring-porous in the initial years, whereas (García-González and Fonti 2008) did not use ca. 25 rings at the beginning of the series to avoid juvenile wood. Yet, the removal of age trend is necessary before computing chronologies through the process known as 'standardization' (Cook et al. 1992), and this also apply to anatomical series (Carrer et al. 2015). As earlywood vessel size appears to practically have no age trend, it can be handled with very stiff or even deterministic trend lines, e.g. a generalized exponential or Hugershoff

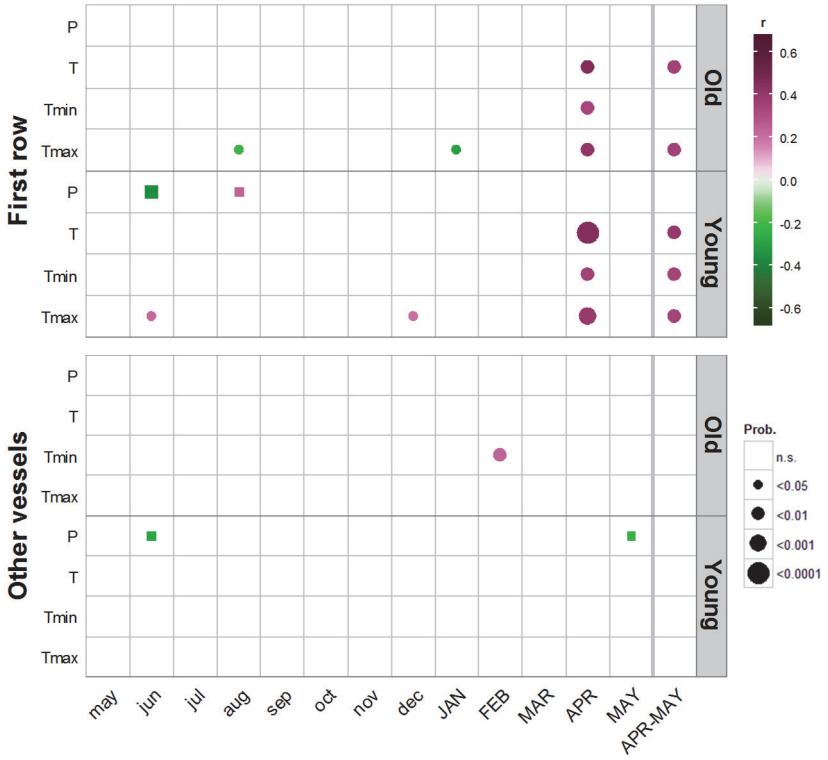

Fig. 9. Relationships to climate for the chronologies of hydraulic diameter (HYD) calculated using bootstrapped correlations, for first row vessels, and for the other vessels, along the study period 1938-2003. Lowercase and uppercase letters correspond to the months of the previous and current growth year, respectively.

function (Warren 1980); and consequently, a flexible spline as that used in most works (Fonti and García-González 2008; García-González and Eckstein 2003; Gea-Izquierdo et al. 2012; González-González et al. 2014). But although the analysis of trend was only related to cambial age, individual series and chronologies showed the absence of abrupt growth changes within HYD series, again confirming that the standardization method should not be an issue limiting the analysis of earlywood vessels. However, Fonti et al. (2009b) found that the detrending method induced frequency-dependent signals for the earlywood vessels of oaks.

Another important aspect to consider when analyzing earlywood anatomy from a dendrochronological perspective is the selection of variables, which is usually performed by comparing the relationships among each other (Fonti and García-González 2004; González-González et al. 2014; Kniesel et al. 2015), or in relation to climatic factors (González-González et al. 2014; González-González et al. 2015). In this case, we analyzed vessel size, expressed as HYD, separating the first row of vessels, after having verified that the variables were split in two groups, namely i) size variables for all vessels and all variables related to the first row, and ii) variables determined by the number of vessels and those calculated from vessels not in the first row. This result was also found by González-González et al. (2014) for two different oak species in northern Spain. Separating into rows is a common practice, because not all earlywood vessels bear the same signal (García-González and Fonti 2006), and it is often optimized by the first elements (Fonti et al. 2007; Gea-Izquierdo et al. 2012; González-González et al. 2014), but this is not always the case (González-González et al. 2015). Therefore, one question that could arise is if the relationships among variables can be related to other parameter such as ring width, be site-dependent, or even age dependent.

In this paper, we tested whether these relationships were related to age, by first comparing both age classes, and then by analyzing the variations among variables through time, and verified that the relations among variables were very robust. For the same time span (1938-2003), we found no differences between *Young* and *Old*. On the other hand, the relationship among variables did not basically change through time. This was observed for the ordination of all variables, but more in detail for the comparison between HYD-r1 and HYD-nr1, whose correlation was highly significant and nearly constant through time. In fact, we only find a few variations, mainly concentrated during the second half of the 19th century; this period presented abundant narrow rings, which caused enormous difficulties during crossdating. In general, narrow rings at this site did not have latewood, but a many rings still presented two rows of vessels, instead of a single one, which can explain the stability of the relations between vessel rows.

Chronology quality and climate-growth relationships

Tree-ring chronologies of earlywood vessels have a very low common signal in most cases, and always much lower than ring width (Fonti et al. 2007; García-González and Eckstein 2003; Gea-Izquierdo et al. 2012; González-González et al. 2014), and considering more vessels or selecting a subset does not increase their values (García-González and Fonti 2006; García-González and Fonti 2008), even if the climatic response can be considerably improved (García-González and Fonti 2008; González-González et al. 2014). However, common signal of HYD-r1 chronologies was considerably higher than usual, probably due to the mountain location of the study site, which imposes some constraints to growth; in this sense, LW chronologies had optimal values of common signal. Furthermore, the variable most commonly employed in this kind of investigations is mean vessel area, and not HYD, which appears to provide an even lower common signal than other earlywood features.

The higher common signal observed for *Young* in this common period, coupled with the greater abundance of small vessels previously described, is probably the most important difference related to age we found; in young vigorous trees, rings are wider and include more vessel rows. However, the temporal analysis for

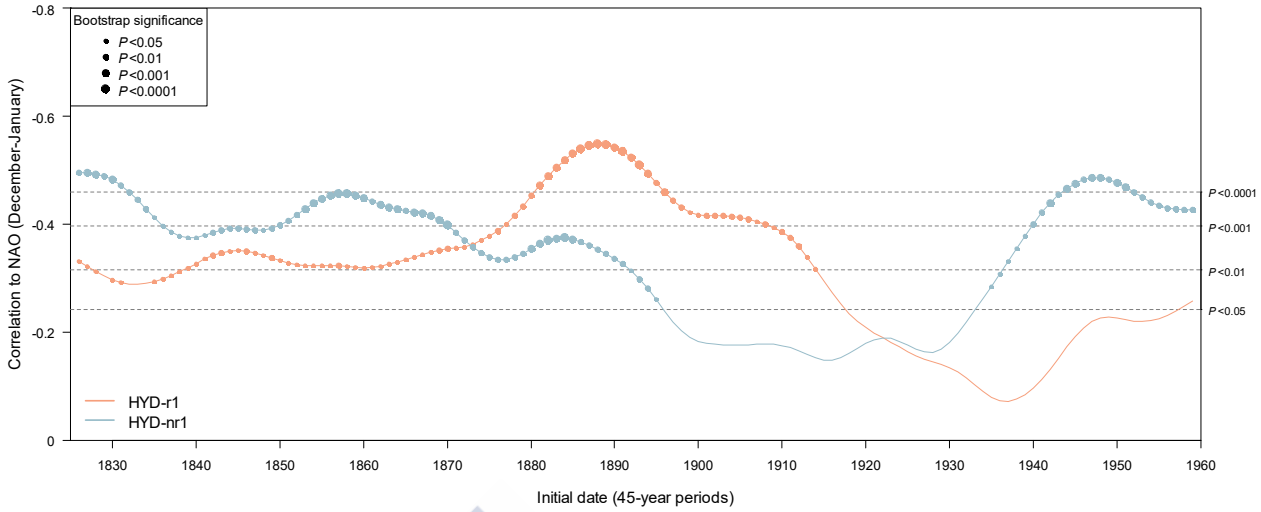

Fig. 10. Variation through time of the bootstrapped correlations between NAO (December-January) and the chronologies of hydraulic diameter for *Old*, calculated on a 45-year moving window along the period 1825-2003. Trend lines were smoothed using spline interpolation. *HYD-r1*: hydraulic diameter for vessels in the first row; *HYD-nr1*: hydraulic diameter for the other vessels.

Old (1626-2003) did not show a descending trend along with age, but fairly constant common signal and interannual variability. Most changes appeared to be related to growth limitations in different periods rather than ageing. We observed an increase in common signal during the 18th century, and a decreasing trend along the 19th century followed by an abrupt change at the end of this period. This was coupled to the presence of narrow rings until the second half of the 19th century. The long period of reduced growth corresponds to the last 100 to 150 years of the Little Ice Age (Mann et al. 1998), probably coinciding with a more dense forest than nowadays; an important deforestation took place after this period due to charcoal elaboration for iron factories (Balboa de Paz 2014), which resulted in a growth recovery all along the 20th century, including the common period for *Young* and *Old*. In our opinion, this trend is probably related to both climate and stand structure, but there is not enough information about forest history or effects of the Little Ice Age in the region to explain our results.

Climate-growth relationships for HYD confirmed again the analogy between *Young* and *Old*, as differences were negligible. Though slightly weaker for *Old*, probably due to the lowest common signal and less vessel rows, they let us assume that there are no differences between age classes. In both cases, only the first row was related to climate, and the fact that young trees included more vessel rows, and consequently a higher proportion of vessels not located in the first row, did not impact the results. Trees exhibited a positive correlation between HYD-r1 and spring (April) temperature, which seems to be related to the breaking of dormancy and the development of the first vessels. Therefore, the effect of climate is direct on earlywood formation, by firing the onset of growth or by modulating the rates of vessel differentiation. Unfortunately, there are no works dealing with xylogenesis at this high-elevation area, but previous surveys from lower inland areas in the region point out that cambial divisions and expansion have already started in April (González-González et al. 2013; Pérez-de-Lis et al. 2015; Souto-Herrero et al. 2005).

Temperature is the main primary factor related to variation in xylem growth, so that cool spring temperatures would delay the onset of cambial cell division, resulting in a shorter duration of the growing period (Prislan et al. 2013). Therefore, a high spring temperature in this mountain area would anticipate wood formation, resulting in a longer season, and

larger vessels. According to Pérez-de-Lis et al. (2015), an early onset of vessel formation results in larger conduits in *Q. robur*.

This mechanism that explains climate-growth relationships for the earlywood vessels appears to be operating nearly independent of age. However, wood formation can be affected by age, as Rossi et al. (2008) stated that cambial activity was delayed for old trees, and it spanned for 2-3 weeks less. But unlike other authors, who found notable differences in the response of trees of different age (Carrer and Urbinati 2004; Szeicz and Macdonald 1994), our results were very similar for both age groups. Our results apparently suggest that the climate control of earlywood vessel size is not age-dependent, but we cannot affirm it as rule, because it is a single work carried out in a mountain environment, which is probably playing more control on growth than could be found at more benign sites. In addition, the pattern of age-dependent sensitivity of trees can be complex (Rozas et al. 2009a).

These analyses of climate-growth relationships only confirm the analogy between *Young* and *Old* at present, but the evaluation of earlywood vessel size as a proxy requires the study of the responses through time. As no temperature records were available, we use the North Atlantic Oscillation (NAO), which is available for a much longer period (Luterbacher et al. 2001). Winter and spring conditions in the western Iberian Peninsula are to a great extent controlled by the NAO, whereby a warm and rainy winter-early spring is associated to a negative value (Trigo et al. 2004). In fact, relationships to winter NAO have already been found for tree-ring chronologies in NW Iberia (Rozas et al. 2009b). The analysis along nearly two centuries showed an important association between NAO index and HYD, as both vessel rows exhibited a negative relation to NAO (i.e., a negative index resulted in larger vessels). But unfortunately this relationship did not prove stable through time, being only highly significant until the beginning of the 20th century. Trends also differed between rows, with a strong relation between NAO and HYD-nr1 starting from the second half of the 20th century. In this preliminary study, we cannot establish if the time instability is due to the behavior of the trees, or because role of NAO on local climate is variable through time. This is coherent with the local results obtained by (Rozas and García-González 2012), who found non-stationary relations between LW chronologies of *Q. robur* and the Southern Oscillation Index (SOI), for NW Spain; and also with the existence of more generalized processes, such as the 'divergence' from the second half of the 20th century (D'Arrigo et al. 2008), i.e., the instability of climate-growth relationships over wide areas. But the existence of a response for a long time period opens the possibility of further exploring the use of earlywood vessels to study long-term climatic trends using different climatic indices.

To our knowledge, we presented in this paper the longest chronology of earlywood vessel size available so far, spanning 1523-2003 (481 years). We studied this chronology along time, and compared it to younger trees. Our results suggested that ageing is not a limitation for the use of earlywood vessel traits as a climatic proxy, since young and old trees do respond to the same climatic factors. Despite a certain recent instability, we also identified a strong relationship to NAO for a long time period. Therefore, though limited to a single site, our work points out the promising potential of earlywood vessels as climatic proxies, and suggest that future research should focus on continuing these guidelines.

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Discusión general

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5 DISCUSIÓN GENERAL

5.1 METODOLOGÍA DE PREPARACIÓN DE CORES Y ANÁLISIS DE IMAGEN

Durante el desarrollo de esta Tesis Doctoral se ha establecido un protocolo metodológico que aglutina todos los procesos desde la preparación de los *cores* hasta la obtención de las medidas derivadas de los elementos conductores. Como innovación, se han desarrollado herramientas que optimizan varias fases del trabajo en las que la eficiencia descendía notablemente, suponiendo un considerable avance en el campo de la dendrocronología aplicada al estudio de la anatomía del xilema.

La utilización del micrótomo de deslizamiento (Gärtner & Nievergelt 2010) para conseguir una superficie plana en los *cores* ha supuesto una notable mejora con respecto al rebajado manual con cuchilla llevado a cabo originalmente, ya que con este último era frecuente el bloqueo repentino del elemento de corte en la madera al rebajar un grosor excesivo, ocasionando hendiduras muy difíciles de corregir en la posterior fase de lijado y, en ocasiones, provocando el desgarro de los vasos o la rotura de la muestra, además de una superficie no homogénea. Se ha observado además que un lijado agresivo, necesario en ocasiones para eliminar estas marcas, acarrea otros problemas indirectos, ya que produce una ligera curvatura de la superficie de las muestras hacia los bordes. Esto hace que el enfoque varíe lo suficiente como para distorsionar esas zonas, entorpeciendo la fase de detección y produciendo una pérdida de anchura útil y vasos, así como una reducción de la calidad de las mediciones. Con el micrótomo, sin embargo, se consigue un corte muy limpio, uniforme y perfectamente horizontal, evitando las marcas del corte en la madera, de modo que el lijado posterior es muy somero ya que está solamente dirigido a mejorar la visualización de los vasos y no a corregir defectos. Debido a que la profundidad de campo de una lupa es muy limitada, el hecho de que la superficie resultante sea completamente horizontal representa otra gran ventaja ya que dicha limitación deja de ser relevante. Además del considerable ahorro de tiempo, con esta técnica se consigue un notable incremento de la calidad de la preparación final.

La tinción de la superficie de cada *core* con tinta negra de impresora (Fonti et al. 2009) también ha presentado ventajas con respecto al método inicialmente utilizado, en el que se empleaba azul astra para oscurecer las células parenquimáticas (sobre todo los radios leñosos) y una disolución de safranina en alcohol al 8 % para teñir la lignina. Aunque de este modo se conseguía una buena tinción de todo el tejido sin dificultar la observación de la estructura anatómica, el contraste obtenido con los lúmenes de los vasos rellenos de polvo de tiza no era suficiente en numerosas ocasiones. A menudo aparecían radios gruesos deficientemente teñidos que dificultaban la detección automática de los vasos, y eran apreciables igualmente las diferencias de tonalidad entre distintas partes de la madera, como la existente entre albura y duramen. En ocasiones también se preparó la superficie del core con sosa diluida al 10% y se taponaron los elementos conductores con cera blanca según las indicaciones de Sass and Eckstein (1994), pero los resultados obtenidos no presentaron la calidad requerida. La utilización de la tinta no solo supone un óptimo contraste sino que además uniformiza el color de fondo de toda la muestra, algo necesario para agilizar la fase de detección. Aunque se pierda la apreciación de la estructura anatómica, un lijado con papel de grano fino (P1200) elimina fácilmente la capa teñida del *core* ya que la penetración de la tinta en la madera es muy superficial, pudiendo recuperar de nuevo el aspecto original para cualquier otro estudio en el que no se requiera o no sea conveniente la presencia del tinte.

El disponer de muestras con un óptimo contraste entre vasos y tejido, con un fondo uniforme y una superficie perfectamente horizontal, ha sido crucial y ha condicionado el desarrollo posterior del dispositivo automático de fotografía *SIFAU*. El éxito en su utilización depende directamente de la calidad final de los *cores*, constituyendo una considerable contribución metodológica en la investigación de los vasos primaverales. Dicha herramienta consta de un dispositivo de microfotografía y una plataforma móvil, integrados y coordinados mediante un software específico llamado *ServoFotos*, que permite fotografiar secuencialmente los *cores*. Por un lado se ha aumentado la rapidez notablemente, ya

que se ha pasado de requerir desde varios días hasta una semana para fotografiar manualmente una localidad (dependiendo este tiempo de la longitud y dificultad de los *cores*) a poder llevarlo a cabo en poco más de una hora y de una forma mucho más organizada, con menores posibles fuentes de error. Por otro lado, se ha incrementado también la eficacia, ya que el proceso manual inducía a cometer numerosos errores, en su mayoría provocados por lo tedioso y repetitivo del trabajo (zonas de solape insuficientes o ausentes, desajustes de enfoque, diferencias de luminosidad en la muestra, etc.) que hacía necesario repetir algunas de las fotografías de la secuencia correspondiente, con la consiguiente pérdida de tiempo.

La simplicidad en el montaje de sus componentes y la utilización de materiales y elementos disponibles en el mercado para cualquier usuario hacen de este dispositivo una interesante alternativa, muy adaptable a las necesidades que en cada momento puedan ir surgiendo y muy versátil a la hora de implementar nuevos componentes, sustituir algún elemento o, simplemente, mejorar sus prestaciones.

Con el desarrollo de esta herramienta se completa la automatización de todas las fases de análisis de imagen. Su uso combinado con *VesselJ* (plugin de detección automática de vasos para el programa generalista *ImageJ*) y con *AutoVasos* (software de datación de vasos y anillos), ambos también de reciente creación, supone una extraordinaria mejora en cuanto a la cantidad de material que se puede procesar y a la calidad de los resultados, ya que se minimizan las probabilidades de error en cualquiera de las fases y de existir alguno, las correcciones pertinentes son relativamente rápidas y fáciles de aplicar. La ventaja de disponer de un pluggin específico frente al uso general del programa *ImageJ* es clara, ya que permite acceder a las herramientas y opciones necesarias para procesar este tipo de material de un modo más rápido y directo. Además, los datos resultantes son salvados con un formato específico que facilita su posterior tratamiento.

Existen otras herramientas específicas de análisis de imagen similares a *VesselJ*. *ROXAS* (Wegner et al. 2013) y *WINCELL* (Regent Instruments Inc., Canada) son dos software desarrollados específicamente para medir las células de la madera tanto en *cores* como en rodajas procedentes de angiospermas y coníferas. Pero en ambos casos su uso está restringido comercialmente, ya que *ROXAS* (de distribución libre) depende del software comercial Image-Pro Plus v.6.17 (Media Cybernetics). Además, en *ROXAS* el tamaño máximo de imagen en cualquier dimensión está limitado a 32.768 pixels. *VesselJ*, sin embargo, depende de un programa libre y de uso gratuito, y la limitación de tamaño de las imágenes, aunque afectó durante la realización de esta Tesis, a fecha actual está ya superada.

5.2 VARIABLES DE CRECIMIENTO Y ANATÓMICAS. RELACIONES Y UTILIDAD COMO PROXIES *CLIMÁTICOS*

Los distintos análisis realizados han permitido establecer las diferencias en la información aportada por cada una de las variables estudiadas en la presente Tesis Doctoral. Las relaciones existentes entre ellas han mostrado ser muy robustas a lo largo del tiempo, no dependientes de la edad y bastante constantes con independencia de la especie, arrojando resultados muy en concordancia con los de trabajos previos (González-González et al. 2014; Kniesel et al. 2015). Se observó con una clara separación entre las variables de los vasos de primera fila, relacionadas con el tamaño, y las del resto de vasos, relacionadas con la densidad de elementos conductores. Estas relaciones han sido más homogéneas a lo largo de las localidades de *Quercus pyrenaica*, presentando grupos mucho mejor diferenciados (Art. IV) que en el caso de *Q. robur*, con mayor dispersión de variables (Art. III). El motivo parece estar relacionado con la menor duración de la estación de crecimiento en *Q. pyrenaica* respecto a la observada en *Q. robur* (Pérez-de-Lis et al. 2015) ya que la fenología de la primera especie está programada para evitar las heladas tardías y para hacer frente a las condiciones de sequía estival (Sánchez de Dios et al. 2009). En contraposición, un período activo más largo unido a una mayor heterogeneidad de los ambientes estudiados puede ser la causa de que en *Q. robur* más que conjuntos de variables se adivinen tendencias, ya que la masa estudiada a mayor altitud (CAB) y con un período activo

considerablemente más corto que el resto también presenta una buena discriminación de grupos de variables.

En vista de las relaciones observadas, a lo largo de la Tesis lo más razonable ha sido estudiar el tamaño de vasos de la madera temprana, utilizando las variables área media de vasos (MVA) y diámetro hidráulicamente ponderado (HYD) tanto para el total de vasos medidos como separándolos por filas, mientras que el crecimiento radial se ha caracterizado a través de la anchura del leño tardío (LW) y la anchura total del anillo (RW). Aunque la variable de madera temprana más habitualmente utilizada en este tipo de estudios es MVA (García-González & Eckstein 2003; Fonti & García-González 2004; Fonti et al. 2007), en varios de los Artículos se ha utilizado HYD ya que ha demostrado ser más robusto debido a que está menos afectado por los vasos pequeños que contribuyen escasamente a la conductividad. A pesar de que ambas variables están estrechamente correlacionadas entre sí y han mostrado grandes similitudes en cuanto a calidad estadística y respuestas al clima al contrastar los resultados, se ha observado una ligera diferencia entre ellas. Mientras MVA parece ser un mejor indicador para diferenciar condiciones locales (Art. I y II), los efectos del clima a lo largo de un área geográfica más amplia han sido reflejados con mejor resolución por parte de HYD (Art. III y IV). Este comportamiento probablemente esté ligado a esa mayor robustez observada ya que en la captación de señales meso- y macroclimáticas es probable que sean los vasos de mayor tamaño los más sensibles y que la inclusión de vasos pequeños en los análisis introduzca cierto "ruido" en la respuesta. No obstante, los distintos análisis llevados a cabo acerca del comportamiento de ambas variables no son suficientes para poder establecer con seguridad cuál de ellas funciona mejor como *proxy* climático, o para determinar en qué ámbitos conviene utilizar una u otra, por lo que conviene llevar a cabo más estudios al respecto en el futuro.

Independientemente de la variable de tamaño utilizada, los vasos primaverales de ambas especies han demostrado ser muy sensibles a señales micro- (Art. I y II), meso- (Art. I-V), y macroclimáticas (Art. IV y V), y en el caso de *Q. robur* han sido también capaces de expresar diferencias topográficas aparentemente relacionadas con las condiciones edáficas y no tanto con el clima local (Art. III). Una ventaja añadida es que son mucho menos propensos que las variables de anchura a registrar los efectos que causan perturbaciones tales como intervenciones humanas, incendios o plagas forestales, tal y como se ha podido comprobar en muchas de las localidades de *Q. pyrenaica* muestreadas (Art. IV), y que pueden llegar a enmascarar las respuestas climáticas captadas por el incremento radial, dificultando su análisis e interpretación (Rozas 2005). Los vasos de *Q. robur*, en especial los pertenecientes a la primera fila, mostraron además una notable estabilidad temporal de la calidad estadística y de la señal común mucho mayor que las variables de anchura, y además dicha calidad estadística no está afectada por la edad de los árboles (Art. V). Estos resultados ponen de manifiesto el gran interés que encierran los vasos para ser utilizados como *proxies* para llevar a cabo reconstrucciones climáticas, ya que han demostrado ser muy robustos y consistentes en el tiempo y el espacio.

5.3 RELACIONES CLIMA-CRECIMIENTO

El estudio de las relaciones clima-crecimiento pone de manifiesto que las variables de madera temprana en ambas especies responden a las condiciones ambientales en tres momentos clave: i) durante la estación de crecimiento anterior (Art. III), ii) durante el período de dormición-quiescencia (Art. I y IV) y iii) al inicio de la estación de crecimiento (Art. II). También han demostrado ser independientes de la edad, a la vista de los resultados obtenidos en el Art. V. La mayoría de las respuestas encontradas están relacionadas con la temperatura, mientras que los efectos más importantes relativos a la precipitación se concentran durante la dormición y la estación de crecimiento. En general se ha observado una optimización de las señales climáticas al analizar los vasos separados por filas, pero esta separación de los vasos según el momento de su formación no parece funcionar tan bien para *Q. pyrenaica*. En la mayoría de los casos analizados la señal recogida por los vasos de la primera fila es

expresada con prácticamente igual intensidad por el total de vasos medidos, por lo que la ganancia de información es mínima, e incluso se ha observado en un caso particular la total pérdida de la respuesta al analizar los vasos por separado (Art. II). Para identificar exactamente qué tipo de vasos registraban la señal climática, en este artículo ha sido necesario recurrir además a los filtros progresivos de tamaño, que han demostrado una enorme utilidad en dicha optimización de las respuestas. Únicamente en el estudio de los efectos de la Oscilación del Atlántico Norte (NAO), donde hay correlaciones significativas con los vasos no pertenecientes a la primera fila, es el caso en el que el total de vasos enmascara la respuesta, siendo entonces necesario el uso independiente de las filas para poder identificarla (Art. IV).

Las respuestas registradas durante la estación de crecimiento anterior son débiles y menos frecuentes; en el caso de *Q. robur* parecen tener cierta relevancia únicamente en las localidades situadas a altitudes medias, mientras que en *Q. pyrenaica* se registran respuestas en ambos extremos altitudinales. Se manifiestan principalmente como una correlación negativa con la precipitación y positiva con la temperatura del mes de Mayo, todas ellas con un bajo nivel de significación y para ambas especies. En la localidad de *Q. robur* situada en el norte de Galicia (MER) tienen cierta influencia negativa también las precipitaciones del mes de Agosto previo al crecimiento. Trabajos anteriores ya han puesto de manifiesto la existencia de estas relaciones con la estación de crecimiento anterior (García González 2000; González-González et al. 2014).

En altitudes bajas en las que predominan inviernos suaves (Martínez Cortizas et al. 1999), y en las que ambas especies se encuentran en sus respectivos límites de distribución, son principalmente las temperaturas mínimas durante la dormición-quiescencia las que regulan el desarrollo de los primeros vasos de la estación de crecimiento. Las especies de anillo poroso forman estos primeros elementos vasculares a partir de derivadas cambiales que se dividen antes de la época de dormición, diferenciándose en la estación siguiente. Así, la primera hilera de vasos puede formarse días o incluso semanas antes de la apertura de las yemas (Suzuki et al. 1996; González-González et al. 2013; Takahashi et al. 2013; Pérez-de-Lis et al. 2015), por tanto a partir de reservas acumuladas durante la estación anterior. En *Q. robur* se han registrado intensas respuestas negativas durante del período Diciembre-Febrero tanto en la costa norte de Galicia (Art. III) como en las zonas de influencia mediterránea del interior (Art. I), intensificadas en este último caso por condiciones locales de exceso hídrico durante el invierno. Esta relación con el invierno, ya observadas en los vasos primaverales por González González et al. (2015), han sido vinculadas con los niveles de reserva y/u hormonales durante el período de dormición y con el hecho de que altas temperaturas en esta época producen una considerable degradación de carbohidratos de reserva para mantener la respiración, quedando menos cantidad disponible para formar los vasos de la primera fila. Las masas de *Q. pyrenaica* situadas a baja altitud (Art. IV), aunque mostraron el mismo comportamiento durante el mes de Febrero, las respuestas en este caso y según las observaciones de fenología cambial efectuadas por Pérez-de-Lis et al. (2015) tendrían lugar durante la quiescencia y no durante la dormición. En esta fase las células cambiales sufren cambios estructurales y hormonales que las predisponen a responder más tarde en la estación activa. Por tanto, temperaturas elevadas en este momento favorecerían una respuesta tardía de los árboles, que quizás unido también a un cierto consumo de carbohidratos por respiración, favorece la formación de vasos más pequeños en estos entornos.

En situaciones de montaña el efecto del invierno se disipa debido a las bajas temperaturas por efecto de la altitud (Martínez Cortizas et al. 1999), pasando a ser determinante para el crecimiento el aumento de las temperaturas mínimas registradas a principios de primavera. Para ambas especies el mes con mayor peso en esta señal es Abril, con un efecto beneficioso de las condiciones cálidas sobre el desarrollo de los vasos de la primera fila. Esta respuesta parece estar ligada a la necesidad de alcanzar un umbral mínimo de temperatura durante la primavera para poder reiniciar la actividad cambial, mientras que a altitudes menores parece estar más condicionado por el fotoperiodo, de acuerdo con las observaciones fenológicas llevadas a cabo para *Q. robur* (Art. III) durante la realización de la Tesis. En el caso de *Q. pyrenaica*, ésta podría ser también la explicación de su comportamiento. Aunque se carece de

datos fenológicos es probable que sea la estrategia adoptada en las zonas más bajas de su distribución para evitar las heladas tardías (Art. IV), mientras que el umbral de temperatura es alcanzado más tarde a altitudes superiores como las registradas en A Gudiña (Art. IV) y Sanabria (Art. II), una vez que los requerimientos de fotoperiodo ya han sido satisfechos, y aparentemente en el mismo momento a pesar de la diferencia altitudinal entre ambas zonas. Las relaciones entre el tamaño de vasos y las condiciones durante la estación de crecimiento ya han sido observadas con anterioridad para la temperatura (García-González & Eckstein 2003; Fonti et al. 2010) A pesar de que es decisivo el papel de esta variable climática a lo largo de la zona de estudio, ambas especies han reflejado también los efectos de la precipitación tanto en la estación de crecimiento (Fonti & García-González 2008) como en la dormición (Rozas & García-González 2012; González González et al. 2015).

Las respuestas a la precipitación están relacionadas fundamentalmente con fenómenos de ámbito local relacionados con el exceso hídrico del suelo, en dos entornos de influencia mediterránea que presentan una importante recarga hídrica durante el invierno (Martínez Cortizas et al. 1999). En *Q. pyrenaica* las condiciones de encharcamiento debidas a la elevada pluviometría registrada en el entorno del Lago de Sanabria (Art. II) parecen afectar produciendo una ralentización del crecimiento primaveral, que se traduce en un retraso en la respuesta a las condiciones ambientales debido a la falta de oxígeno en las raíces; por otro lado la duración del encharcamiento en cada una de las localidades muestreadas por efecto de las condiciones microtopográficas marca claras diferencias entre ellas relacionadas con el tipo de vasos que expresan dicha señal. En *Q. robur* los efectos nocivos del encharcamiento se han detectado con mucha intensidad a baja altitud, en una masa del interior de Galicia sometida a sequía estival acusada y temperaturas invernales suaves (Martínez Cortizas et al. 1999) debido a la influencia mediterránea (Art. I), bajo condiciones edáficas tendentes una rápida saturación de agua. En este caso los vasos de la primera fila han respondido negativamente al exceso hídrico durante la dormición, época en la que ya existía un consumo de carbohidratos de reserva debido a la respiración por las altas temperaturas, y al que se suma el adicional requerido para evitar el impacto negativo de la hipoxia radicular (Vartapetian & Jackson 1997). Estos procesos afectan por tanto al tamaño de los vasos primaverales.

Los efectos de la NAO, cuyas variaciones condicionan el clima regional durante el invierno y gran parte de la primavera, fueron registrados por los vasos primaverales de ambas especies, pero con especial intensidad en aquellos territorios en los que se ha observado una mayor influencia de la teleconexión climática sobre el clima local (Figura S.2; Art. IV). Así, *Q. robur* solamente registró respuestas significativas en las Montañas Orientales de Galicia (Art. V), mostrando una correlación negativa de todos los vasos medidos con la NAO de invierno, relación ya observada con anterioridad en el Noroeste de la Península Ibérica con las cronologías de anchura (Rozas et al. 2009). Esta señal además se ha mantenido prácticamente estable durante 120 años hasta mediados del siglo XX. A diferencia de la anterior especie, *Q. pyrenaica* recoge la influencia de este parámetro macroclimático a lo largo de un transecto en el entorno de las Montañas Surorientales de Galicia (Art. IV). Siguiendo un claro patrón altitudinal, las respuestas negativas han sido registradas a finales de invierno en las localidades más bajas y por los vasos de la primera fila, a principios de primavera en las localidades de altitud media pero a través de los vasos formados más tarde en la estación, y perdiendo vigencia en las zonas de mayor altitud. Este resultado es coherente con el hecho de que la NAO no parece ejercer efecto sobre el clima peninsular a partir de finales de la primavera. La intensidad de las señales registradas y la existencia de una respuesta sostenida a lo largo de un período largo de tiempo hace posible utilizar los vasos primaverales de ambas especies de roble para llevar a cabo estudios climáticos más complejos, así como posibles análisis de tendencias climáticas a largo plazo.

La madera tardía recogió respuestas en general menos intensas y diferentes para cada especie. En el caso de *Q. robur* la disponibilidad hídrica estival como factor limitante se ha apreciado de manera directa en el norte de Galicia, respuesta ya identificada con anterioridad por García-González and Eckstein (2003) y Rozas (2005), pero también en las localidades del interior sometidas a sequía estival, donde el efecto es visible únicamente a mayor escala temporal (Art. I). En el resto de localidades,

situadas en un entorno montañoso (Art. III), la ausencia de respuestas a las condiciones de verano probablemente esté relacionado con un menor estrés hídrico debido a temperaturas más bajas y al frecuente aporte de lluvias por tormentas de tipo convectivo (Martínez Cortizas et al. 1999). Lo que sí parece ser un patrón general en la región, aunque no demasido acentuado, es un cierto pulso de crecimiento favorecido por temperaturas cálidas durante el mes de Septiembre, detectado en casi todas las localidades muestreadas. Para *Q. pyrenaica* sin embargo, el incremento radial está determinado principalmente por la disponibilidad hídrica entre finales de la primavera y principios del verano. Esta respuesta se recoge de manera muy uniforme a lo largo de todas las localidades muestreadas en esta Tesis, confirmando que el principal factor que parece controlar el crecimiento radial del rebollo en la Península Ibérica es la precipitación de verano (Gea-Izquierdo & Cañellas 2014).

El análisis espacio-temporal de los vasos primaverales ha contribuido al mejor conocimiento de las especies *Quercus robur* y *Quercus pyrenaica*, y esto puede repercutir positivamente a la hora de entender/inferir su comportamiento futuro ante diversos escenarios como el cambio climático.

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Conclusiones generales

6

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6 CONCLUSIONES GENERALES

La presente Tesis Doctoral, realizada a partir de la medición de 647.979 vasos primaverales de *Quercus robur* y *Q. pyrenaica*, provenientes de 165 árboles distribuidos en 13 localidades, ha permitido extraer las siguientes conclusiones:

1. Los vasos de madera temprana de estos robles presentan una notable respuesta al clima, la cual permite su uso como indicadores o *proxies*. Esta respuesta se registra principalmente en su tamaño, y está condicionada a una correcta selección que tenga en cuenta las dimensiones y la posición de los mismos.

2. Las variables derivadas de los vasos primaverales resultan eficientes registrando condiciones micro-, meso- y macroclimáticas. La extracción de esta información sólo puede lograrse mediante el estudio de los diferentes compartimentos de cada anillo y su variación temporal, junto con la variación espacial a lo largo de diversos ambientes.

3. La completa comprensión de los factores ambientales que afectan a la formación de madera a lo largo de la estación activa sólo puede lograrse combinando el estudio de madera temprana y tardía por separado. El análisis de la madera temprana debe realizarse a partir de variables anatómicas de los elementos conductores, mientras en la madera tardía resulta fundamental el estudio del incremento radial.

4. El análisis combinado de madera temprana y tardía permitió identificar las principales variables ambientales que controlan el crecimiento de estos robles en el noroeste de la Península Ibérica. Mientras las características anatómicas de la madera temprana parecen estar fuertemente relacionadas con el clima, el incremento radial puede estar notablemente afectado por el régimen de perturbaciones de cada masa forestal.

5. En situaciones de influencia mediterránea existe un mayor control ejercido por las condiciones climáticas. A baja altitud tiene lugar un notable efecto sobre el balance de reservas durante la dormición, que se ve potenciado por una mayor limitación para la asimilación en la época de crecimiento activo.

6. *Q. pyrenaica* presenta una mayor respuesta al clima, probablemente por estar ligado a ambientes más restrictivos, así como por presentar un período vegetativo más reducido. Por el contrario, *Q. robur* sólo muestra respuestas claras en torno a su límite de distribución.

7. La metodología seguida en este trabajo mostró que es posible obtener cronologías de gran longitud a partir de los elementos conductores primaverales, abarcando casi cinco siglos. Dado que las relaciones clima-crecimiento de los vasos no parecen variar con la edad del árbol, éstos podrían constituir un robusto *proxy* para estudios retrospectivos.

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